GE Healthcare

ImageMaster 2D Platinum 6.0

User Manual

Contents

1 Introduction

1.1 About ImageMaster 2D Platinum 1
1.2 What's new in this release? 1
1.3 ImageMaster resources 2
1.3.1 User manual 2
1.3.2 Tutorials 2
1.3.3 Online help 2
1.3.4 Conventions used 3
1.4 Getting further assistance 3
1.4.1 How to contact us 3
1.4.2 Sending e-mail 5
1.4.3 System and product information 5
1.4.4 GE Healthcare listens 5

2 Two-dimensional gel analysis

2.1 Two-dimensional electrophoresis 7
2.1.1 Technique 7
2.1.2 Applications 7
2.1.3 Advantages and limitations 7
2.2 Differential gel electrophoresis 8
2.2.1 Technique 8
2.2.2 Internal standard 10
2.3 Understanding gel images 11
2.3.1 Anatomy of gel images 11
2.3.2 Spot and gel relationships 12
2.4 Image analysis workflow 13
2.4.1 Acquiring data 13
2.4.2 Setting up a workspace 13
2.4.3 Visualizing and calibrating gels 14
2.4.4 Detecting and quantifying spots 14
2.4.5 Annotating spots and pixels 14
2.4.6 Matching gels 14
2.4.7 Analyzing data 14
2.4.8 Integrating data 14
2.4.9 Reporting results 15
2.4.10 Controlling and automating gel analyses 15
2.5 ImageMaster flavors 15

3 Getting started

3.1 Installing ImageMaster 2D Platinum 17
3.1.1 System requirements 17
3.1.2 Software installation 17
3.2 eLicensing 18

3.2.1 Access code ... *18*
3.2.2 Finding the physical address of the computer ... *18*
3.2.3 Installing the GE Healthcare eLicense server ... *19*
3.2.4 Collecting and placing an eLicense file ... *20*
3.2.5 Test the eLicense ... *21*
3.3 Starting and exiting ImageMaster ... 21
3.4 ImageMaster window ... 22
3.4.1 Menu bar ... *23*
3.4.2 Toolbar ... *24*
3.4.3 Display zone ... *24*
3.4.4 Dockable windows ... *28*
3.5 ImageMaster options ... 30

4 Workspace

4.1 Introduction ... 33
4.2 Workspace window ... 35
4.2.1 Workspace toolbar ... *36*
4.2.2 Active project ... *36*
4.2.3 Navigator ... *37*
4.2.4 File details ... *37*
4.3 Setting up a workspace ... 37
4.3.1 Creating a workspace ... *38*
4.3.2 Opening an existing workspace ... *38*
4.3.3 Workspace properties ... *38*
4.4 Working with projects ... 39
4.4.1 Creating a project ... *39*
4.4.2 Inserting an existing project ... *40*
4.4.3 Exporting a project ... *40*
4.4.4 Project properties ... *40*
4.5 Working with gels ... 41
4.5.1 Creating a subfolder ... *41*
4.5.2 Importing a new gel ... *42*
4.5.3 Importing a new DIGE gel ... *43*
4.5.4 Adding an existing gel ... *43*
4.5.5 Adding an existing DIGE gel ... *44*
4.5.6 Moving gels or folders ... *44*
4.5.7 Displaying gel images ... *44*
4.5.8 Spot detection ... *45*
4.5.9 Folder properties ... *45*
4.5.10 Gel properties ... *45*
4.6 Working with match sets ... 46
4.6.1 Creating a match set ... *49*
4.6.2 Creating a match set from a DIGE gel ... *50*
4.6.3 Adding gels to a match set ... *51*
4.6.4 Copying match sets ... *51*
4.6.5 Setting the match set reference ... *52*
4.6.6 Moving gels or match sets ... *52*
4.6.7 Displaying match sets ... *52*

4.6.8 Matching ... *53*
4.6.9 Match set properties ... *53*
4.7 Working with classes ... 54
4.7.1 Creating a class ... *56*
4.7.2 Adding gels to a class ... *57*
4.7.3 Moving gels or classes ... *58*
4.7.4 Displaying classes ... *58*
4.7.5 Statistical analysis ... *58*
4.7.6 Class properties ... *58*
4.8 Working with reports ... 59
4.8.1 Creating a subfolder ... *59*
4.8.2 Adding reports ... *59*
4.8.3 Opening reports ... *60*
4.8.4 Moving reports or folders ... *60*
4.9 Working with documents ... 60
4.9.1 Creating a subfolder ... *60*
4.9.2 Adding files ... *61*
4.9.3 Opening documents ... *61*
4.9.4 Moving documents or folders ... *61*
4.10 Gel and report identifiers ... 61
4.11 Archiving a workspace ... 62
4.11.1 Backup / Restore workspace ... *62*
4.11.2 Backup / Restore project ... *63*

5 Reports

5.1 Introduction ... 65
5.1.1 Reports menu ... *65*
5.1.2 Analyze menu ... *66*
5.2 Report toolbars ... 67
5.2.1 Standard tools ... *67*
5.2.2 Adding comments ... *68*
5.2.3 Selecting and navigating ... *68*
5.2.4 Displaying related reports ... *70*
5.3 Editing report content ... 72
5.4 Customizing reports ... 74
5.4.1 Settings ... *74*
5.4.2 Column order ... *75*
5.4.3 Column and row size ... *75*
5.4.4 Sorting data ... *76*
5.5 Mouse selection reports ... 76

6 Gels

6.1 Introduction ... 79
6.1.1 Image format ... *79*
6.1.2 Image resolution ... *79*
6.1.3 Image depth ... *80*
6.2 Selecting gels and gel regions ... 80
6.3 Displaying gels ... 82

6.3.1 Moving gels ... *82*
6.3.2 Zoom gels ... *83*
6.3.3 Scrollbars ... *87*
6.3.4 Transparency ... *88*
6.3.5 Grid lines ... *90*
6.3.6 Aligning gels ... *92*
6.4 Viewing signal intensity ... 94
6.4.1 Loading / unloading gels ... *94*
6.4.2 Cursor information ... *95*
6.4.3 Displaying gray levels ... *96*
6.4.4 Contrast mapping ... *96*
6.4.5 Profile ... *101*
6.4.6 3D view ... *102*
6.5 Calibrating and normalizing gels ... 106
6.5.1 Intensity calibration ... *106*
6.5.2 Spot normalization ... *111*
6.5.3 Intensity normalization using a scatter plot ... *112*
6.6 Processing gels ... 114
6.6.1 Delete from disk ... *114*
6.6.2 Renaming gels ... *114*
6.6.3 Rotating gels ... *115*
6.6.4 Flipping gels ... *116*
6.6.5 Scaling gels ... *117*
6.6.6 Inverting gray levels ... *117*
6.6.7 Cropping gels ... *118*
6.7 Reporting on gels ... 119
6.7.1 Gel report ... *119*
6.7.2 Gel description report ... *120*
6.7.3 Gel calibration report ... *122*
6.8 Saving, exporting or printing images ... 123
6.8.1 Saving gels ... *123*
6.8.2 Exporting gels and windows ... *124*
6.8.3 Printing gels ... *125*

7 Spots

7.1 Introduction ... 127
7.2 Spot ID ... 127
7.3 Detecting spots in non-DIGE gels ... 128
7.3.1 Procedure ... *128*
7.3.2 Spot detection parameters ... *130*
7.3.3 Spot quantification ... *131*
7.4 Co-detecting spots in DIGE gels ... 133
7.4.1 Procedure ... *133*
7.4.2 Spot detection parameter ... *134*
7.4.3 Spot quantification ... *134*
7.5 Selecting spots ... 135
7.5.1 Spot tool ... *135*
7.5.2 Select menu ... *135*

7.5.3 Reports ... 136
7.6 Displaying spots ... 136
7.6.1 Spot shape ... 136
7.6.2 Spot color ... 137
7.6.3 Show / hide spots ... 137
7.6.4 Show / hide spot IDs ... 138
7.7 Adding / modifying spots ... 138
7.7.1 Editing spots ... 139
7.8 MW and pI calibration ... 142
7.9 Cursor information ... 143
7.10 Reporting on spots ... 145
7.10.1 Spot Report ... 145
7.10.2 DIGE histogram ... 148
7.10.3 DIGE report ... 150

8 Annotations

8.1 Introduction ... 151
8.2 Predefined label categories ... 151
8.3 Creating annotations and labels ... 152
8.3.1 Creating label categories ... 154
8.3.2 Creating sets ... 158
8.3.3 Creating specific links ... 158
8.4 Selecting annotations and labels ... 161
8.4.1 Annotation tool ... 161
8.4.2 Select menu ... 162
8.4.3 Reports ... 167
8.5 Displaying annotations and labels ... 167
8.5.1 Annotation flag position ... 167
8.5.2 Annotation flag color ... 167
8.5.3 Annotation flagpole color ... 168
8.5.4 Show / hide annotations and labels ... 168
8.5.5 Pack / unpack categories ... 170
8.6 Adding / modifying annotations and labels ... 171
8.6.1 Annotation tool ... 171
8.6.2 Edit menu ... 171
8.6.3 Reports ... 174
8.6.4 Importing labels and annotations ... 175
8.7 Annotations and labels in reports ... 176
8.7.1 Label report ... 176
8.7.2 Annotation report ... 177
8.7.3 Category report ... 177
8.7.4 Intra-Class Report ... 177
8.7.5 Inter-Class Report ... 178

9 Matches

9.1 Introduction ... 179
9.2 Matching ... 181
9.2.1 Automatic matching ... 181

9.2.2 Specifying starting matches ... 182
9.3 Selecting matches ... 183
9.3.1 Menu options ... 183
9.3.2 Reports ... 186
9.4 Displaying matches ... 186
9.4.1 Show / hide matches ... 187
9.4.2 Show / hide match IDs ... 187
9.5 Adding / deleting matches ... 188
9.5.1 Adding / deleting matches ... 188
9.6 Reports on matches ... 189
9.6.1 Match report ... 189
9.6.2 Match statistics report ... 190
9.6.3 Intra-class report ... 190
9.7 Synthetic gels ... 191
9.7.1 Spot filtering ... 192
9.7.2 Creating synthetic gels ... 193

10 Data analysis

10.1 Introduction ... 195
10.2 Intra-class statistics ... 196
10.2.1 Scatter plots ... 196
10.2.2 Descriptive statistics ... 198
10.2.3 Intra-Class Histograms ... 201
10.2.4 Intra-Class Report ... 205
10.2.5 Factor analysis ... 209
10.2.6 Heuristic clustering ... 214
10.3 Inter-class statistics ... 216
10.3.1 Specifying classes ... 216
10.3.2 Overlapping measures ... 216
10.3.3 Inter-Class Histograms ... 220
10.3.4 Inter-Class Report ... 224
10.3.5 Statistical tests ... 225

11 Data integration

11.1 Converting into ImageMaster 2D Platinum format ... 233
11.1.1 From earlier versions of ImageMaster and Melanie ... 233
11.1.2 From ImageMaster 2D Elite ... 233
11.1.3 From Twain compatible scanners ... 236
11.2 Exporting and importing ImageMaster 2D Platinum data ... 237
11.2.1 XML format ... 237
11.2.2 ImageMaster reports ... 238
11.2.3 Export gel data ... 238
11.2.4 Import gel data ... 240
11.3 Exporting to spot excision robots ... 241
11.3.1 Bruker Proteineer SP spot picker ... 241
11.3.2 GE Healthcare Ettan spot picker ... 241
11.3.3 Genetix GelPix spot picker ... 244
11.3.4 Genomic Solutions ProPic spot picker ... 244

11.4 Connecting to external protein databases 245
11.4.1 Setting the database 245
11.4.2 Querying the database 246
11.4.3 SWISS-2DPAGE master gels 246

12 Safety, control and automation

12.1 Introduction 249
12.1.1 Action descriptors 249
12.1.2 Displayed actions 249
12.1.3 History and script navigators 250
12.1.4 Gel selection 251
12.2 Undo / redo 251
12.3 History 253
12.4 Script 255

A Appendix

A.1 Contextual menus 259
A.2 Workspace contextual menus 259
A.2.1 Workspace name 259
A.2.2 Project name 259
A.2.3 Gels folder 259
A.2.4 DIGE Gels folder 261
A.2.5 MatchSets folder 262
A.2.6 Classes folder 263
A.2.7 Reports folder 264
A.2.8 Documents folder 265
A.3 Display zone contextual menus 266
A.3.1 Worksheet, pane or legend 266
A.3.2 Image 266

B Appendix

B.1 Shortcut keys 269
B.2 Gel shortcuts 270
B.3 Spot shortcuts 271
B.4 Annotation shortcuts 272
B.5 Match shortcuts 273

C Appendix

C.1 Software references 275
C.2 Statistical methods 276
C.3 Further reading 276

Contents

1 Introduction

1.1 About ImageMaster 2D Platinum

ImageMaster 2D Platinum is a state-of-the-art software application designed to analyze two-dimensional electrophoresis (2-DE) gels. This version of ImageMaster has been developed by a team of top researchers from the Swiss Institute of Bioinformatics (SIB) in collaboration with Geneva Bioinformatics (GeneBio) SA and GE Healthcare, and integrates the Melanie software. It was conceived in close collaboration with biologists and proteomics scientists from Professor Hochstrasser's group in Geneva. The application has advanced to its current level primarily due to the input of users worldwide.

ImageMaster 2D Platinum ensures fast and reliable image comparisons. It easily manages multiple image analyses and offers the possibility to automate detection and matching steps. However, it is the versatility of the software that empowers you, the scientist. There are numerous interactive tools for optimizing and manipulating data. Furthermore, attain a higher level of quantitative and qualitative analysis using the robust and sophisticated techniques provided in the application. ImageMaster integrates filtering, querying, reporting, statistical and graphing options so that you can easily view, compare, analyze and present your results. From the raw experimental images to the preparation of a publication, ImageMaster takes you successfully through each stage of an image study.

1.2 What's new in this release?

The most important new features in ImageMaster 2D Platinum, version 6.0 are listed below:

- Population matching
- Support of DIGE technology (co-detection, DIGE histograms)
- Intelligent multi-worksheet display
- Dockable report windows
- Workspace with management of gels, match sets and classes
- Scrollbars for moving and zooming gels
- Improved cropping option
- Adjust contrast in 3D View
- Measure histogram in spot report

1.3 ImageMaster resources

1.3.1 User manual

The ImageMaster 2D Platinum software, incorporating Melanie, is intended to be intuitive and comprehensive. We encourage you to read through the User Manual in order to exploit its full potential. This guide explains in detail all the functions and advantages of ImageMaster 2D Platinum. If new to the software, you will quickly master the fundamental concepts and features. If already familiar with the software, the manual will inform you of new functionalities and help to optimize your image analyses.

The chapters in this manual are generally organized according to the logical sequence of a 2-DE gel analysis, although expert users will agree that many of the steps can be inverted or repeated at some point. The last chapters are more specialized and have no defined place in the general procedure of an image analysis study.

1.3.2 Tutorials

You can familiarize yourself with ImageMaster 2D Platinum by working through the online tutorials. These tutorials offer a step-by-step guide on how to analyze your gel images and report results. They are supplied with gel images and related files, enabling you to carry out an entire image analysis using the various tools available in the software.

You can access the different tutorials from the *Help* menu in the main program window. For each of the tutorials, the option *Help* > *Tutorials* > *Tutorial X* > *Open* will prepare your environment, restore the necessary files, and display the tutorial text. The option *Help* > *Tutorials* > *Tutorial X* > *Result* shows the expected outcome after running the corresponding tutorial.

1.3.3 Online help

The user manual is also available online from within the ImageMaster software.

To consult the online manual:

1 Choose *Help* > *User Manual* from the menu.

2 ImageMaster opens the online document.

3 Find the desired section from the *Contents*, *Index* or through a *Search*.

1.3.4 Conventions used

Before getting started with ImageMaster, you should have a working knowledge of your computer's operating system and its conventions, including how to use a mouse and standard menus and commands. You need to be able to adeptly open, save and close files. If you wish to review any of these skills, please refer to the documentation that came with your computer.

Convention	Use
Bold	Highlights different options or parameters that are inherent to ImageMaster 2D Platinum. It also draws attention to newly discussed terms.
Italic	Highlights menu options, window names, icon names, file paths and folder names.
Italic > *Italic*	The arrow indicates a menu choice. For example, you would choose the italicized menu title and then click on the italicized option.
NOTE!	Highlights important comments, issues or tips.
Shift	Designates holding down the Shift key.
Ctrl	Designates holding down the Control key.
Left click	Means clicking the left mouse button.
Right click	Means clicking the right mouse button.
Double click	Means clicking the left mouse button twice.
Drag	Signifies positioning the cursor on an object and holding down the left mouse button while moving the mouse.

Table 1-1. Conventions used in the ImageMaster documentation.

1.4 Getting further assistance

GE Healthcare provides technical and scientific support for the ImageMaster software. Please contact us if any problems arise with the installation or use of the program. Our support team is happy to help you.

1.4.1 How to contact us

E-mail us at imagemaster-support@ge.com.

Support numbers:

Country/Region	Tel	Fax
Asia Pacific	+852 2811 8693	+852 2811 5251
Australasia	+ 61 2 9899 0999	+61 2 9899 7511
Austria	01/57606-1619	01/57606-1627
Belgium	0800 73 888	03 272 1637
Canada	1 800 463 5800	1 800 567 1008
Central, East, & South East Europe	+43 1 982 3826	+43 1 985 8327
Denmark	45 16 2400	45 16 2424
Finland & Baltics	+358-(0)9-512 39 40	+358 (0)9 512 39 439
France	01 6935 6700	01 6941 9677
Germany	0761/4903-490	0761/4903-405
Italy	02 27322 1	02 27302 212
Japan	+81 3 5331 9336	+81 3 5331 9370
Latin America	+55 11 3933 7300	+55 11 3933 7306
Middle East & Africa	+30 210 9600 687	+30 210 9600 693
Netherlands	0165 580 410	0165 580 401
Norway	815 65 555	815 65 666
Portugal	21 417 7035	21 417 3184
Russia & other C.I.S. & N.I.S	+7 (095) 232 0250 +7 (095) 956 1137	+7 (095) 230 6377
South East Asia	60 3 8024 2080	60 3 8024 2090
Spain	93 594 49 50	93 594 49 55
Sweden	018 612 1900	018 612 1910
Switzerland	0848 8028 12	0848 8028 13
UK	0800 616928	0800 616927
USA	+1 800 526 3593	+1 877 295 8102

1.4.2 Sending e-mail

To provide us with the essential technical and customer information when mailing to imagemaster-support@ge.com, please proceed as follows:

To send an e-mail to technical support:

1 Choose *Help* > *Online Support* from the menu.

2 Enter your personal details in the *Customer Information* box. Please note that this data is used to update our customer database. In this way, we can contact you with certainty when it is time to send new patches or installation files.

3 In the *Online Support* box, specify whether you want to ask a question, make a suggestion, or report a bug. Depending on your choice, the software asks you subsequent questions that help us to supply you with an appropriate answer.

4 Click on the *E-mail* button. A new message is created with your default e-mail software, automatically directed to imagemaster-support@ge.com. Highlight the <Paste clipboard> text with your mouse and then *Edit* > *Paste* the contents of your clipboard into the mail message. The e-mail now contains your questions and contact information, as well as useful data about your ImageMaster version, work session and computer system. If the problem is file specific, then attach any relevant files. Send us this message. Alternatively, you can print the clipboard contents and send it to us by fax.

1.4.3 System and product information

To quickly obtain product and system information, for your own benefit or during a technical support call, choose *Help* > *System & Product Information* from the menu. A window shows the useful data about your ImageMaster version and computer system.

You can also get information about your ImageMaster version by looking at the release number given under *About ImageMaster* in the *Help* menu. Choosing *Help* > *About ImageMaster* displays the program splash page.

1.4.4 GE Healthcare listens

The GE Healthcare staff is attentive to your suggestions. Many of the new features and enhancements in this software are a direct result of conversations with our customers. We truly appreciate any comments, criticisms or ideas that would help us to improve the software. Please do not hesitate to contact us by e-mail at imagemaster-support@ge.com.

2 Two-dimensional gel analysis

2.1 Two-dimensional electrophoresis

2.1.1 Technique

Two-dimensional electrophoresis (2-DE) can effectively resolve thousands of proteins in a complex biological mixture by separating the proteins based on two independent properties: isoelectric point (pI) and molecular weight (MW).

Separation in the first dimension is achieved by re-hydrating an immobilized pH gradient (IPG) strip with the protein sample and subsequently applying an electric field. The charged proteins migrate within the pH gradient until they reach their isoelectric point, where they lose their net charge and therefore their mobility. Non-equilibrium pH gradient electrophoresis (NEPHGE) can also be used to accomplish this separation.

After the isoelectric focusing (IEF) step, the strip is placed at the cathodic end of a homogeneous or gradient sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), possibly along with molecular weight marker proteins flanking one or both ends of the IEF strip. In an electric field, the negatively-charged SDS-protein complexes then migrate towards the anode with differing velocities depending on their size. Small molecules move faster and further. Larger ones move more slowly and cover less distance.

The proteins are finally visualized by radiolabeling or detected with a variety of staining methods such as silver, Coomassie blue or fluorescent stains. Adapted image capture devices are used to generate digital images that can be analyzed with a 2-DE image analysis software such as ImageMaster.

2.1.2 Applications

Two-dimensional (2-D) gel electrophoresis is a leading research tool in proteomics. This technique enables the identification of protein expression changes such as those associated with the exposure to pathogens, drugs or other stimuli, or alterations linked to different stages of development. Thus, gel electrophoresis is invaluable in gene expression studies for the discovery of protein targets, disease markers, and drug candidates, or to evaluate drugs for toxicity. And this is just to name a few examples.

2.1.3 Advantages and limitations

2-DE is currently the most powerful protein separation technique, allowing the parallel investigation of thousands of proteins in a single step. The technology is compatible with almost all protein types. Gels with narrow pH ranges permit very

high resolution and large protein loads so that even poorly expressed proteins can be observed.

However, scientists wanting to compare a set of gel images may notice that the quality and reproducibility of 2-D gels are sometimes mediocre. Poor resolution, high noise levels, and large distortions in the protein patterns often hamper the analysis process. The reasons for this are extensive: unskilled sample preparation leads to unintended biological variability, poor sample solubilization produces insufficient resolution, vertical and horizontal streaking caused by salt in the IPG strip makes proper detection or quantification difficult, dust particles or droplets lead to many spot artifacts, etc.

The crucial algorithms in 2-D gel analysis software have been significantly improved over the years in order to account for such difficulties. In addition, program interfaces have gained a lot of ground in user-friendliness. In spite of these advances, no image analysis software can solve all experimental problems. The desire to draw valuable information from low quality gels remains an illusive goal. Before starting a 2-D gel image analysis, it is therefore essential to optimize all steps in the gel production process in order to assure the best possible quality and reproducibility. Careful experimentation remains the only way to guarantee significant end results. This includes refining protocols for the sample preparation of each starting material, pre-fractionating the sample, using precise and correct experimental conditions during 2-D separation and protein staining or labeling, employing larger gel formats or narrower pH ranges, and so on. Image acquisition parameters also play a very important role.

2.2 Differential gel electrophoresis

2.2.1 Technique

Conventional 2-DE techniques require the separation of each sample on an individual gel in order to compare protein abundance in different samples. This one-sample-per-gel approach introduces a high level of system variation to the data such as the variation that arises from differences in protein uptake into the first dimension strip, second dimension gel running, etc. This high level of system variation can outweigh the often subtle, induced biological changes that the experiment is intended to detect, for example, differences that are caused by a disease state, drug treatment or life-cycle stage.

To further complicate this problem, it is also necessary to separate the induced biological changes within an experiment from the inherent biological variation. In other words, the differences between two individual animals, cultures or plants, that are present irrespective of the applied experimental conditions. To achieve this, multiple sample replicates must be incorporated within each experimental

design. This requires the separation and analysis of a large number of samples and slows the process if each sample must be separated on a different gel.

Differential gel electrophoresis (DIGE) is a method for pre-labeling protein samples prior to two-dimensional electrophoresis. By allowing different samples to be run on a single gel, one of which can be an internal standard, it can significantly simplify the above mentioned problems.

The system is based upon the use of up to three cyanine dyes possessing unique fluorescent properties. The dyes (Cy2, Cy3 and Cy5) are mass- and charge-matched N-hydroxy succinimidyl ester derivatives that differentially attach to lysine residues in a protein. Therefore the labeled proteins migrate simultaneously on the 2-DE gel but still produce distinct excitation and emission spectra.

The design of a DIGE experiment is straightforward (Figure 2-1). Protein samples are labeled with the different DIGE dyes and subsequently mixed on the same 2-DE gel. Electrophoresis is applied to separate the proteins according to molecular weight and isoelectric point. The gel is then scanned using instrumentation such as the Typhoon Variable Mode Imager (GE Healthcare) to generate overlaid, multi-channel images for each gel.

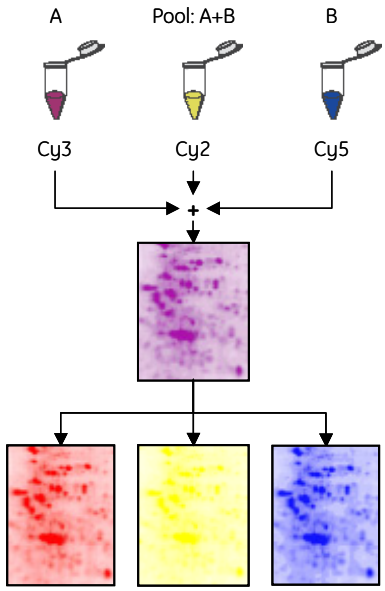


Figure 2-1. Simple scheme for DIGE. Three protein extracts are labeled, combined on one 2-DE gel and then separated according to the fluorescence spectra.

Depending on the amount of sample available, different dye to protein ratios can be used. Minimal labeling with Cy2, Cy3 and Cy5 is most straightforward to use. When only a small amount of sample is available, saturation dyes might be the best choice. The Cy3 and Cy5 saturation dyes are also mass and charge matched, except that they are maleimide fluors that react with thiols found on cysteine residues to form a thioether.

Keep in mind that if no lysine or cysteine amino acid (which are targeted by the minimal and saturation dyes, respectively) is present in a protein, the protein is undetectable.

2.2.2 Internal standard

This type of methodology allows an internal standard to be included. Ideally, the internal standard consists of a subset taken from all of the samples within the experiment. The internal standard is labeled with one of the cyanine dyes (Typically Cy2 for minimal dyes and Cy3 for saturation dyes) and run on each gel in the experiment. This creates an image that is the average of all experimental samples, with all proteins in the experiment represented. The presence of the internal standard in every DIGE gel provides an inherent link between samples.

It is the use of an internal standard in 2-DE experiments that permits quantitative and statistical analyses. First, each protein spot in a sample can be compared to its representative spot within the internal standard on the same gel, to generate a ratio of relative protein levels. Quantitative comparisons of samples between gels are made based on the relative change of sample to its in-gel internal standard. This process effectively removes the system (gel-to-gel) variation enabling accurate quantitation of induced biological change between samples. The need to run gel replicates is also eliminated, thus reducing the number of gels required per experiment.

An additional benefit of using an internal standard in DIGE technology is that matching between gels is more apparent. The internal standard image is common between all gels in an experiment, therefore matching can be performed between internal standard images which have similar spot patterns. Conventional 2-DE requires matching between different samples on different gels, which introduces differences in spot patterns from sample-to-sample and gel-to-gel variation. Matching between internal standards allows matching between identical samples, so variations in spot patterns are due only to electrophoretic differences.

2.3 *Understanding gel images*

2.3.1 Anatomy of gel images

Gels

A gel is a 2-DE image that has been digitized and stored on a drive. It contains the raw input data from which proteins can be detected and quantified. The proteins represent the gray values for all the pixels in the image. Every pixel is characterized by its X and Y coordinates, which represent the horizontal and vertical positions of the pixel on the image. The pixel's raw value, or gray value, is the signal intensity of the pixel (Z axis in a three-dimensional view).

The software recognizes gels from several image formats, but the gels must be saved in the ImageMaster file format (.mel) in order to store data about spots, matches, annotations, etc. If a gel is exported with its objects into a foreign format, then the data simply becomes part of the saved image and loses any structure or value.

Gels can be duplicated, deleted, cropped, filtered, flipped or scaled. Gels can also be aligned, meaning that they are distorted in order to superimpose their image with another gel.

Various items can be defined on a gel for detailed examination. These are spots, regions, annotations and labels (Figure 2-2). Matches, match sets and classes are entities that give structure to the relationships between gels (Figure 2-3) and will be discussed in the following section.

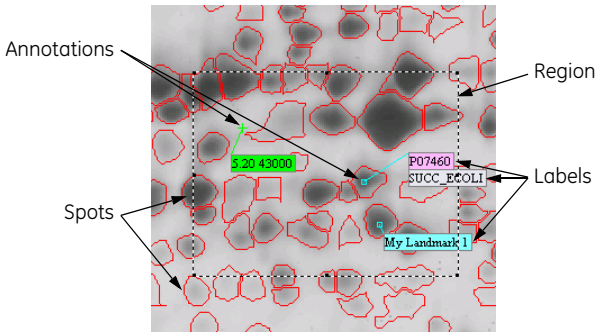


Figure 2-2. Items on a gel: region, spots, annotations and labels.

Regions

A region is a rectangular area of a gel that is defined using the *Region* tool. When a region is set, certain actions may be limited to it, such as previewing spot detection parameters, adjusting gray levels, viewing three-dimensional plots of spot intensities, cropping, etc.

Spots
Spots represent the proteins on the gel. They can be automatically detected by ImageMaster or manually edited by the user. Spots can be quantified. Their intensity, area and volume are computed.

Annotations and labels
Spots or individual points (pixels) can be marked by annotations. An annotation is defined by its position and its set of labels. It contains relevant information that is separated into label categories (predefined and/or user-defined categories).

The predefined categories in ImageMaster include **pI_MW** (holds standard pI and MW values for a pI/MW calibration), **Ac** (contains database accession numbers), **Landmark** (defines tie points for gel warping and matching), **Set** (marks spots that share the same properties) and **Comment** (keeps user information). Moreover, an unlimited number of user-defined categories can be added. Labels can also contain direct links to other files, Web sites and query engines.

2.3.2 Spot and gel relationships

Matches
A match represents the relationship between corresponding spots in different gels. It connects the same protein in the different gels. It is composed of a spot n-tuple (S1, S2, ..., Sn) where S1 is a spot in the first gel, and Sn a spot in the last gel. Matches can be manually defined by the user or automatically determined by ImageMaster's powerful gel matching algorithm. The match is the basic element for searching and investigating protein expression changes across gels, through the use of reports, histograms, and statistical methods.

Match sets
To be able to initiate the matching process in ImageMaster 2D Platinum 6.0, gels must be part of a Match set. A match set includes gels or populations of gels (in sub match sets) that should be compared and therefore matched together. Every match set is represented by a Master image. This master image is created by ImageMaster based on the Reference for the match set, chosen by the user.

Classes
A class is a set of gels or gel populations that you can compare with other such entities. In the Classes folder, you state your biological questions. For example, a class of gels obtained from the infected tissue samples of different patients can be compared to a class of gels representing healthy tissue from other patients.

A class is the basic element for searching and investigating protein expression changes across gels, through the use of intra-class reports, histograms, and scatter plots. You can also compare different classes through inter-class analyses. To compare gels in a class or within different classes, the gels must be matched together. This means that they must belong to the same match set.

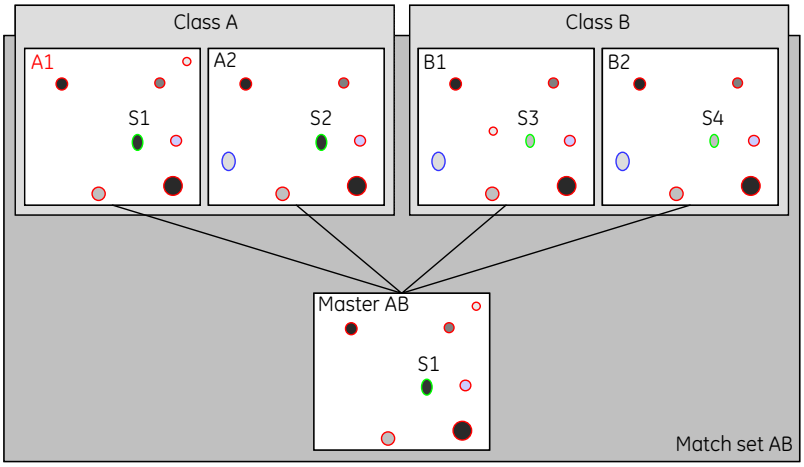


Figure 2-3. Definition of matches, match sets and classes. Spots S1, S2, S3, and S4 together form a match. Note that the spot in Master AB has the same number as the corresponding spot in A1. This is because A1 is the reference image for Match set AB, on which the Master AB is based. The Match set AB contains all the gels to be matched (A1, A2, B1 and B2), and is represented by a master image (Master AB). A class regroups gels from a particular biological state that has to be compared with another such state. The spots in the selected match (green) are under-expressed in Class B with respect to Class A.

2.4 Image analysis workflow

A typical image analysis, both for non-DIGE and DIGE gels, would consist of the following steps. Please note that the specificities of DIGE gel analysis are explained in the corresponding chapters.

2.4.1 Acquiring data

Your gel images must first be digitized using an image capture device. This will generally be done with a separate software. You can open gels from TWAIN compatible scanners from within ImageMaster. Please consult Chapter 11 for more details about this . In Section 6.1 you will also find recommendations on image scanning (format, resolution, depth).

2.4.2 Setting up a workspace

You must set up a workspace to open and work on gel images. The workspace allows you to organize your gels into projects, to define match sets and classes and to keep accompanying data, such as reports and image documents in project related folders. Your preferred ImageMaster settings are also saved in the workspace file. Detailed information about the workspace is found in Chapter 4.

2.4.3 Visualizing and calibrating gels

The next step is to handle the gel files (open, save, print, close), manipulate the gels images (select, move, zoom, stack, align), possibly transform the gels (rotate, crop, scale) and view the signal intensity (adjust contrast, profile, 3-D view). At this stage, it may also be necessary to calibrate your gels. Information about all these features is given in Chapter 6.

2.4.4 Detecting and quantifying spots

Once you are familiar with your gel images, you can perform automatic spot detection. In Chapter 7 you will learn everything you need to know about spots, including how to select, display and edit spots, as well as how to view their properties and quantification values.

2.4.5 Annotating spots and pixels

Individual pixels and spots in a gel image may be labeled with annotations. These annotations can be used in functionalities such as calibration, alignment and matching, or be utilized to mark spots with their particular characteristics. Chapter 8 explains how to create, use, select and display labels, categories and annotations. It further describes how to create links to external databases or data sources of any format (text, file, html, etc.).

2.4.6 Matching gels

After spots were detected and match sets defined, you can match your gel images. In other words you find the corresponding protein spots in different gels. Chapter 9 introduces you to all the necessary concepts and functionalities for gel matching and the creation of synthetic gels.

2.4.7 Analyzing data

Chapter 10 offers an overview of the powerful data analysis and classifications tools that are used to study the variations in protein expression among gels or classes of gels. The data analysis step may be carried out at two different levels. The intra-class statistics tools include scatter plots, descriptive statistics, and factor analysis. For inter-class analyses, the so-called overlapping measures and various statistical tests can be used. Heuristic clustering can help finding classes.

2.4.8 Integrating data

You may want to export spot coordinates to a spot excision robot, export gel data to a database (for example, via XML format), or import experimental information to be included in annotations. The necessary tools are described in Chapter 11 of this manual.

2.4.9 Reporting results

You can display information on specific gels and gel components (spots, matches, classes, annotations) at any moment during your analysis. You will learn the details about displaying, using, saving, customizing and editing reports in Chapter 5. Additionally, you will find information on specific report types in the various chapters of this document.

2.4.10 Controlling and automating gel analyses

As with reporting results, you can check the operations that were carried out on your gels at any time. Do this with the History function that is described in Chapter 12. The latter also instructs you on how to create Scripts for automating parts of your analysis and explains the multiple undo/redo feature.

2.5 ImageMaster flavors

The powerful suite of features that normally apply to 2-DE gels in ImageMaster can now be used in conjunction with DIGE gels. There are two modules of ImageMaster 2D Platinum 6.0 available for purchase:

- **ImageMaster 2D Platinum 6.0**: The software version to be used with conventional 2-DE gels. All menu options related to DIGE are not functional and are grayed out in the graphical user interface.
- **ImageMaster 2D Platinum 6.0 DIGE**: The fully functional version to be used with conventional 2-DE and DIGE gels. You can add and import DIGE gels directly in the workspace. You can also co-detect DIGE gels using the algorithm created by the GE Healthcare DeCyder software development team, match, report, plot histograms and perform statistical analyses on DIGE gels.

3 Getting started

3.1 Installing ImageMaster 2D Platinum

3.1.1 System requirements

In order to install and run ImageMaster, your computer must satisfy the following requirements:

- Microsoft Windows 2000 or XP operating systems.
- Administrative permission to install ImageMaster.
- At least 256 MB RAM for ImageMaster (512 MB is recommended) and 768 MB RAM for ImageMaster DIGE (1024 MB is recommended). The amount of memory required is mainly determined by the number and size of image files to be processed simultaneously. Increased memory therefore enhances the performance when many and/or large images are analyzed.
- A high-quality display. To take full advantage of the software including the 3D View feature, the color resolution should be set to 24 bit (16.7 million colors). However, a color resolution of 8 bit (256 colors) is generally sufficient. It is recommended to use a screen resolution of at least 1024 x 768 pixels.
- At least 60 MB of available disk space for program files and approximately 400 MB for the full installation of tutorials.
- Internet Explorer 6 (Microsoft Corporation), Netscape Navigator 6 (Netscape Communications Corporation), Mozilla 1.4 (Mozilla Foundation) or higher versions. A browser enables you to print reports and to access scientific databases on the Web.

3.1.2 Software installation

You can install ImageMaster from a CD-ROM or by downloading the installation package over the Internet. When you insert the CD-ROM into the appropriate drive on your computer, the Setup Wizard starts automatically and guides you through a series of screens. Alternatively, you can double click on the icon of the installer file (for example SetupIMPv600.msi) to launch the install program.

The ImageMaster installer creates a default directory on your hard disk called *Program Files\GE Healthcare\ImageMaster 2D Platinum*, in which the program files are placed. If you want to save the default directory in a different folder, then browse and open the folder before continuing the installation.

Once installation is complete, it is recommended to restart your computer.

3.2 eLicensing

An electronic license (eLicense) file is required to enable ImageMaster to run once installation is completed.

There are two types of eLicenses:

- **Node-locked license** (Machine license): This type of license is for a single computer. It is practical when only one or a few computers are used for working with ImageMaster. A node-locked license file must be placed on the computer running ImageMaster.
- **Floating license** (Concurrent license): This type of license can be used by all computers connected via network to a computer with a license server installed. It is useful when many users, but not all at the same time, need access to the ImageMaster program. The number of computers that can simultaneously use ImageMaster depends on the license, and is administered via the license server. A floating license file must be placed on the computer running the eLicense server. This server can either be installed on a computer running the ImageMaster program or on any other network computer (It is recommended to install it on a network computer that is always running).

To be able to start ImageMaster, a valid license must be found. ImageMaster will check that the license file is available at every subsequent login. To obtain and correctly place a license file, follow the activation steps below.

3.2.1 Access code

After ordering ImageMaster, a letter including an access code will be send to the order's shipment address. The access code is necessary for collecting the eLicense files (see Section 3.2.4). Store this access code in a safe place.

3.2.2 Finding the physical address of the computer

The physical address is necessary when collecting the appropriate eLicense file. The address identifies the computer and is used by the licensing system. If a node-locked license is used, it is the physical address of the computer where ImageMaster is installed. If a floating license is used, it is the physical address of the computer where the license server is installed.

To find the physical address:

1 On the computer where the eLicense file should be placed, select *Start* > *(All) Programs* > *Accessories* > *Command Prompt*.

2 When the command-prompt appears, click on it and enter the command **ipconfig /all** (making sure to leave a space between the **ipconfig** and the **/all**).

3 Among the displayed information, make a note of the **Physical Address** but without any dashes or colons. If the computer has several Physical Addresses (there can be one for each network, i.e. Ethernet, card in the computer), any of them can be used for identification. Please verify that the Physical Address is correctly noted. Otherwise the license file will not work.

4 Close the command-prompt window.

3.2.3 Installing the GE Healthcare eLicense server

The installation in this section should only be performed when using a floating license. If only node-locked licenses are used, continue from Section 3.2.4.

To install the GE Healthcare eLicense server

1 On the computer where the eLicense server will be installed, insert the ImageMaster 2D Platinum installation CD.

2 Click on the *Install GE Healthcare eLicense Server* button.

3 The installation window is shown. Click *Next*.

4 Accept the default installation path and start the installation (if the default installation path is changed, the paths below need to be updated accordingly).

5 A question concerning the Windows Firewall may appear. To allow the license server to work properly, you must answer *Yes*.

6 At the end of the procedure, ensure that the **Launch GE Healthcare Software Licensing Server** box is checked and click *Finish*. This automatically launches the license server dialog (LMTOOLS).

7 Click the *Config Services* tab and ensure that all options are set as in Figure 3-1. Note that the paths shown (*C:\Program Files\GE Healthcare\eLicense server*) are valid if the default installation is made.

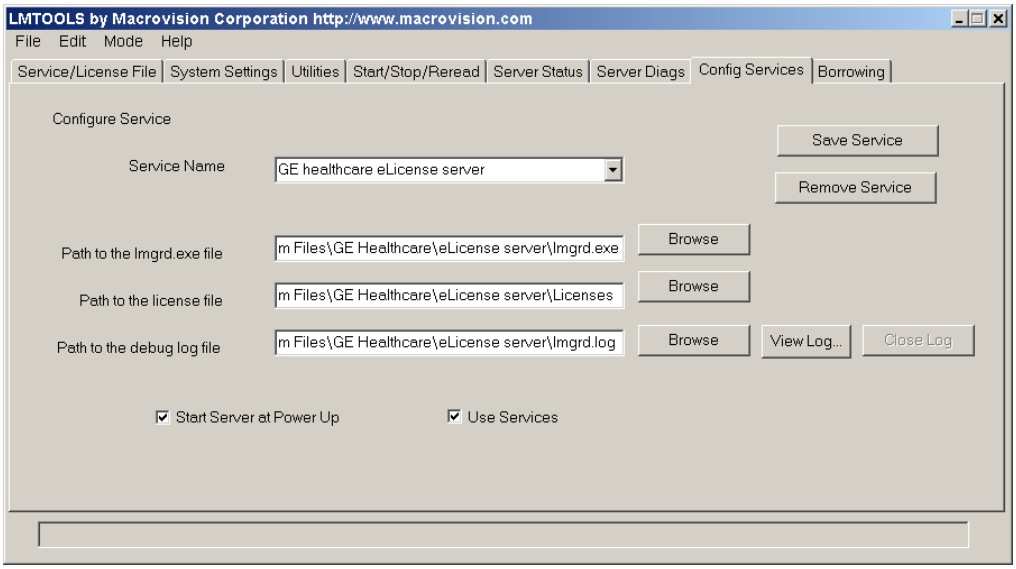


Figure 3-1. Configuration in the Config Services tab in LMTOOLS.

8 Click the *Save Service* button, even if you made no changes in the previous steps.

9 Close LMTOOLS.

3.2.4 Collecting and placing an eLicense file

Next, an eLicense file should be collected and placed in the appropriate folder on your computer. In the case of a node-locked license, the license file should be placed in the ImageMaster installation folder (which is by default *C:\Program Files\GE Healthcare\ImageMaster 2D Platinum*). For a floating license, the file should be in the folder *C:\Program Files\GE Healthcare\eLicense server\Licenses* on the computer running the license server (see Section 3.2.3).

To collect and place a license file:

1 Go to the web site http://www.elicensing.amershambiosciences.com/gtlweb/login.

2 Enter the access code and press *Continue*.

3 Click on *Collect Licenses*.

4 Step 1 - Select the product to collect (mark the check box).

5 Step 2 - Register your product.

6 Step 3 - Enter the **Physical Address** (Host-ID) of the computer where you will install the license file (The expected type of Host-ID shall by default be ETHERNET). Ensure that the Physical Address is correct. Click on *Continue*.

7 If the data shown in the next screen is correct, select to *Collect License*. Otherwise use *Back* to edit.

8 Save the license to a file, or e-mail the license. It is strongly recommended to save the license file. Close the *Download Complete* window after saving the file. If the license is e-mailed, the content is delivered as raw text in the e-mail body. The text must then be copied to an ASCII-text file given the extension .lic.

9 Place the license file in the appropriate folder if this was not already done when saving the license file, or if the license file was e-mailed.

10 Restart the computer.

3.2.5 Test the eLicense

After installation of ImageMaster, downloading and placing the license file, start ImageMaster on all computers where the program is installed to test that the license file can be found (see below).

When using a floating license, a *FLEXlm License Finder* window will be displayed asking to specify the **License Server System** or **License File**. Choose the first option and then enter the name of the computer on which the license server is installed. Click *OK*.

Note that depending on the license acquired (ImageMaster 2D Platinum DIGE or ImageMaster 2D Platinum), you will or will not be able to use the DIGE functionalities in the software.

3.3 Starting and exiting ImageMaster

ImageMaster starts like any other software by selecting *Start* > *Programs* > *GE Healthcare* > *ImageMaster 2D Platinum* from the *Windows* menu. You can also double click the *ImageMaster* icon (Figure 3-2) on the desktop. The ImageMaster splash page appears while the software is loading. You can click on the logo to make it disappear.

Figure 3-2. ImageMaster icon.

Select *File* > *Exit* in the menu to close the program. You can also click on the *Close* button in the upper right corner of the program window. ImageMaster prompts you to save any modifications made to gel images and matching data before exiting. The open workspace will be saved automatically.

3.4 *ImageMaster window*

By reading through the following pages, you will become acquainted with the graphical user interface. It is divided into four main parts, shown in Figure 3-3, which are the **Menu Bar**, the **Toolbar**, the **Display Zone**, and **Dockable Windows**. Each of them will be discussed in more detail below.

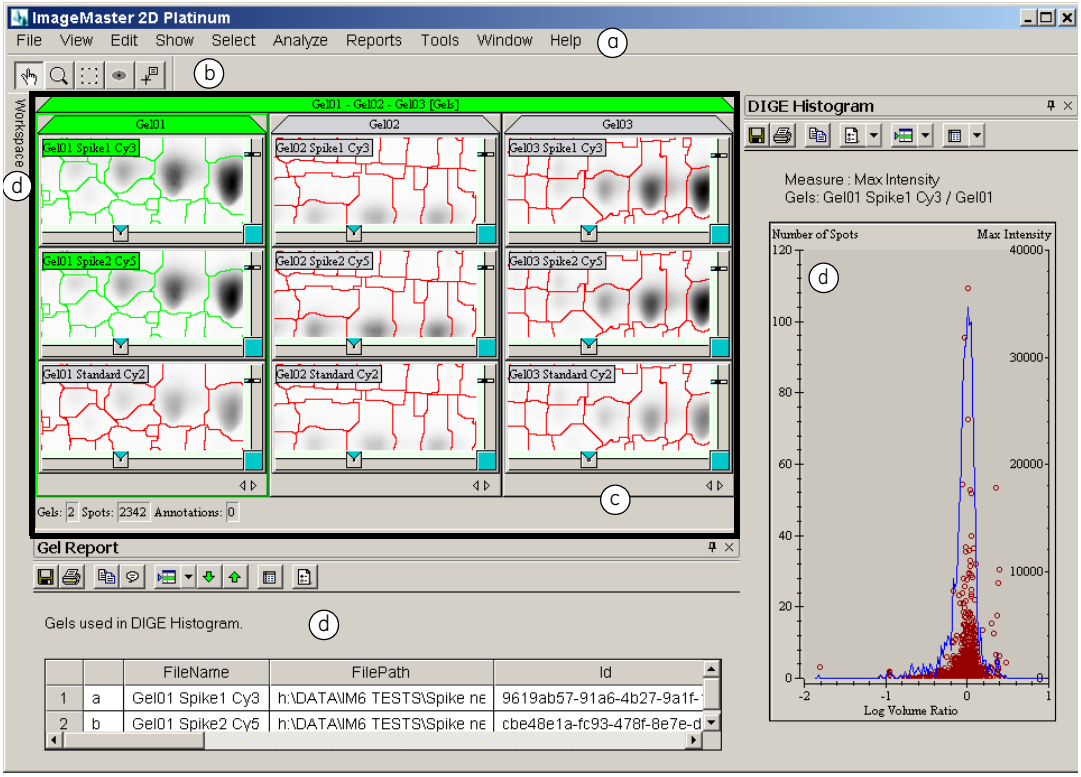


Figure 3-3. The ImageMaster window. (a) Menu Bar, (b) Toolbar, (c) Display Zone and (d) Dockable Windows.

3.4.1 Menu bar

If gels, spots, annotations or matches are selected, then you can choose actions from the menu bar to be performed on them. This is done by choosing the appropriate menu and moving the cursor to the desired option while holding down the left mouse button. Certain menu options have sub-options. You can choose one of these by moving the mouse over the small arrow on the right-hand side of the menu item.

Menu	Usage
File	Perform basic functions such as close, save, import, export and print. You can also exit ImageMaster.
View	Modify the worksheet layout or name. Change the arrangement of images in a pane.
Edit	Undo/redo the last operations, show a history of operations, or edit (add, modify or delete) specific gels, spots, annotations or matches.
Show	Change the way gels are displayed, show/hide specific spots, annotations or matches, or modify their physical properties (shape, color or visibility of IDs).
Select	Select specific gels, spots, annotations or matches based on their ID, value, content or other properties.
Analyze	Compute differences and similarities between gel images (data analysis based on robust statistics, factor analysis, statistical tests and clustering techniques). All reports containing calculated properties are found in this menu.
Reports	Display information on selected gels, spots, labels, annotations, categories or matches, and use the navigation tools in the reports to view the data on your gels. The three-dimensional (3D) view is treated as a report and is therefore located in this menu.
Tools	Remove or rename gels on the hard disk, create new gel images from selected ones (flip, rotate and scale gels, or create synthetic images), run scripts, manage calibrations and change ImageMaster settings.
Window	Display pixel and/or spot information, open zoom windows or mouse selection reports and get a list of all open report windows.
Help	Obtain support online, access the official ImageMaster Web site or obtain information about the current version of ImageMaster.

Keyboard shortcuts

Several menu options can be activated by keyboard shortcuts. These are indicated at the right-hand side of the corresponding menu option. A list of all

shortcuts is given in Appendix B. Please note the logic behind the key combinations:

Ctrl is used to maneuver **gels**.

Shift is used to maneuver **spots**.

Alt is used to maneuver **annotations**.

Ctrl + Shift is used to maneuver **matches**.

3.4.2 Toolbar

Objects on the screen can be displayed, selected or processed using the various options in the ImageMaster menus. However, they can also be manipulated with the tools provided in the toolbar. The fine points of these tools are explained in other sections of the User Manual but the main features are as follows:

	When the **Hand** tool is activated, you can move your image in order to show other parts of it.
	The **Magnify** tool lets you repeatedly zoom in (left click) or out (right click) on the whole image each time by a factor of two.
	The **Region** tool is useful for selecting a rectangular area within an image. This region can be used for cropping gels, selecting objects within a region, or getting a preview zone for spot detection or contrast adjustment.
	The **Spot** tool lets you select or edit individual spots. The tool changes when in the *Edit Enabled* mode.
	The **Annotation** tool is used to add, edit, move or select annotations and labels.

3.4.3 Display zone

Gel images are opened from the Workspace (see Chapter 4 for details) and subsequently displayed in the Display Zone (Figure 3-4). The Display Zone is laid out in three levels: **Worksheets**, **Panes** and **Images**. Their layouts can be customized according to your requirements. A **Status Bar** is located at the bottom of the Display Zone.

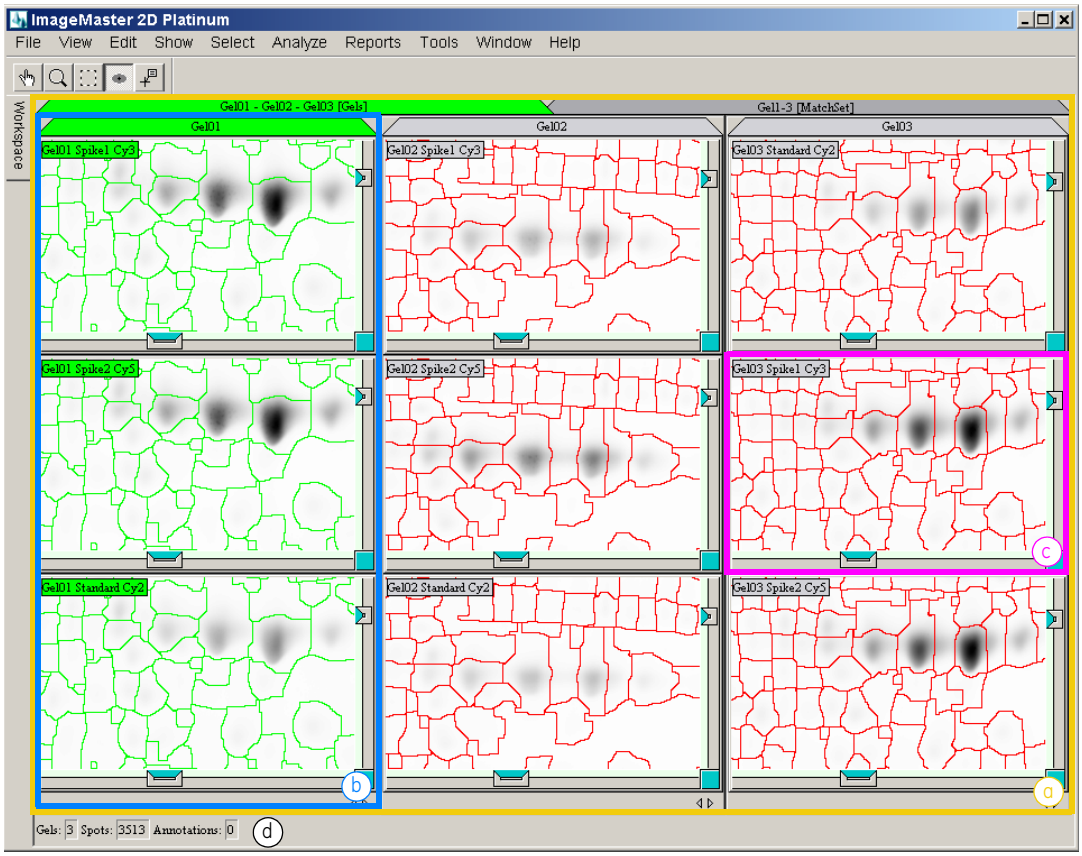


Figure 3-4. The Display Zone. (a) Worksheet, (b) Pane, (c) Image and (d) Status Bar.

Worksheets

Images are always displayed in worksheets. Each worksheet has a banner, which is the horizontal bar located at the top containing the worksheet name and data set type (enclosed in brackets []). The color of the worksheet banner indicates whether it is selected (green) or not (gray). The selected worksheet is always at the front of the Display Zone. In order to select an inactive worksheet, click on its banner. The worksheet is brought to the front of the Display Zone.

To rename a worksheet:

1 Select the worksheet.

2 Choose *View* > *Rename Worksheet* in the menu.

3 In the *Rename Active Worksheet* box, input the name you want. Click *OK*.

4 The new worksheet name appears in the banner.

To close a worksheet:

1 Select the worksheet. If all of its contents are not already selected press Ctrl+A.

2 Choose *File* > *Close Images* in the menu.

3 The worksheet is removed from the Display Zone.

4 In order to close all worksheets at one time, choose *File* > *Close All* in the menu.

Panes

When an image or folder of images is opened in a worksheet, it is automatically placed in a pane. The pane banner is labeled with the same name that was given to the folder of images in the Workspace. Random images are opened in a pane with the generic label Gels. The color of the pane banner indicates whether it is selected (green) or not (gray). In order to select a pane, click on its banner.

To close a pane:

1 Select the pane. If all of its contents are not already selected, then click on its banner.

2 Choose *File* > *Close Images* in the menu.

3 The pane is removed from the worksheet.

A worksheet can contain one or more panes. Panes can be laid out so that they do not overlap (tiled panes) or so they do overlap (stacked panes). Stacked panes resemble a pile of papers lying one on top of another; only the topmost pane is displayed in full. You can move a pane to the top of the stack by clicking on its banner.

By default, ImageMaster displays panes in tiled mode.

To stack panes:

1 Select the worksheet.

2 Choose *View* > *Worksheet Layout* > *Stacked* in the menu.

3 The panes are stacked.

You can also fix the number of panes displayed horizontally and vertically in a worksheet. Choose *View* > *Worksheet Layout* > *Free* in the menu. In the *Worksheet Layout* box, input the following values:

- **Number of Columns**: Constrains the panes positioned one next to the other in the worksheet.
- **Number of Rows**: Constrains the panes positioned one above the other in the worksheet.

Images

A pane can contain one or more gel images. Each image has a legend with its name in the upper left corner. The color of the legend indicates whether the image is selected (green, or red for a master image) or not (gray, or pink for a master image). In order to select an image, click on its legend.

To close a gel image:

1 Select the gel image. Use the Shift or Ctrl keys to make multiple selections.

2 Choose *File* > *Close Images* in the menu.

3 The image is removed from the pane.

Images can be laid out so that they do not overlap (tiled images) or so they do overlap (stacked images). By default, ImageMaster displays images in tiled mode.

To stack images:

1 Select the pane.

2 Choose *View* > *Pane Layout* > *Stacked* in the menu.

3 The images are stacked.

You can also fix the number of images displayed horizontally and vertically in a pane. Choose *View* > *Pane Layout* > *Free* in the menu. In the *Pane Layout* box, input the following values:

- **Number of Columns**: Constrains the images positioned one next to the other in the pane.
- **Number of Rows**: Constrains the images positioned one above the other in the pane.

When the number of open images in a pane exceeds the number of displayed images (for example, in stacked mode) you can make an image visible by clicking on its tab at the bottom of the pane.

In order to sift through images (for example, in stacked mode), use the Page Up and Page Down keys on your keyboard. Alternatively, you can choose *View* > *Pane Layout* in the menu and select *Previous* or *Next*.

Switch order
You can change the order by which the gel images are displayed by clicking on the gel legend and dragging the image onto another gel. It is inserted before this gel. Similarly, you can re-order panes by clicking on their banners and dragging them to their new position.

You can swap worksheets, panes and images. To do so, use *View* > *Switch*, or the Ctrl+F shortcut. This reverses the previous re-ordering operation carried out on a worksheet, pane or image.

Contextual menus
When you click the right mouse button on a worksheet banner, pane banner or gel legend in the Display Zone, a contextual menu appears containing the main options used to work with images, panes or worksheets. If you right click directly on a visible gel image, then another contextual menu offers the possibility to edit, show or select objects like gels, spots, annotations and matches. These short menus duplicate some of the commands found in the menu bar but are ordered differently and are quicker to access at any time. A list of all contextual menus is given in Appendix A.

Status bar
The status bar is an important resource because it indicates the total number of gels, spots and annotations that are currently selected in the active worksheet.

3.4.4 Dockable windows

The workspace and reports are displayed in dockable windows that can be set according to your preferred style of working with numerous windows at a time. Dockable windows can be docked (i.e. fixed) in place against the left, right, top or bottom edges of the ImageMaster window and always lie on top when visible. A visible window can be in **Dockable** (pinned), **Auto Hide** (un-pinned) or **Floating** mode.

Dockable (pinned)
The pinned mode enables dockable windows to be locked into position around the edges of the ImageMaster window. Once a window is pinned, you can move it to a different location by dragging the title bar (Figure 3-5). ImageMaster displays blue arrow guides to indicate where the window may be docked. By

moving the cursor over the guides, a shaded blue box appears showing where the window will reside if the left mouse button is released. If you move the dockable window to a non-predefined location, it becomes a floating window. Moving a docked window may affect the location and size of other docked windows.

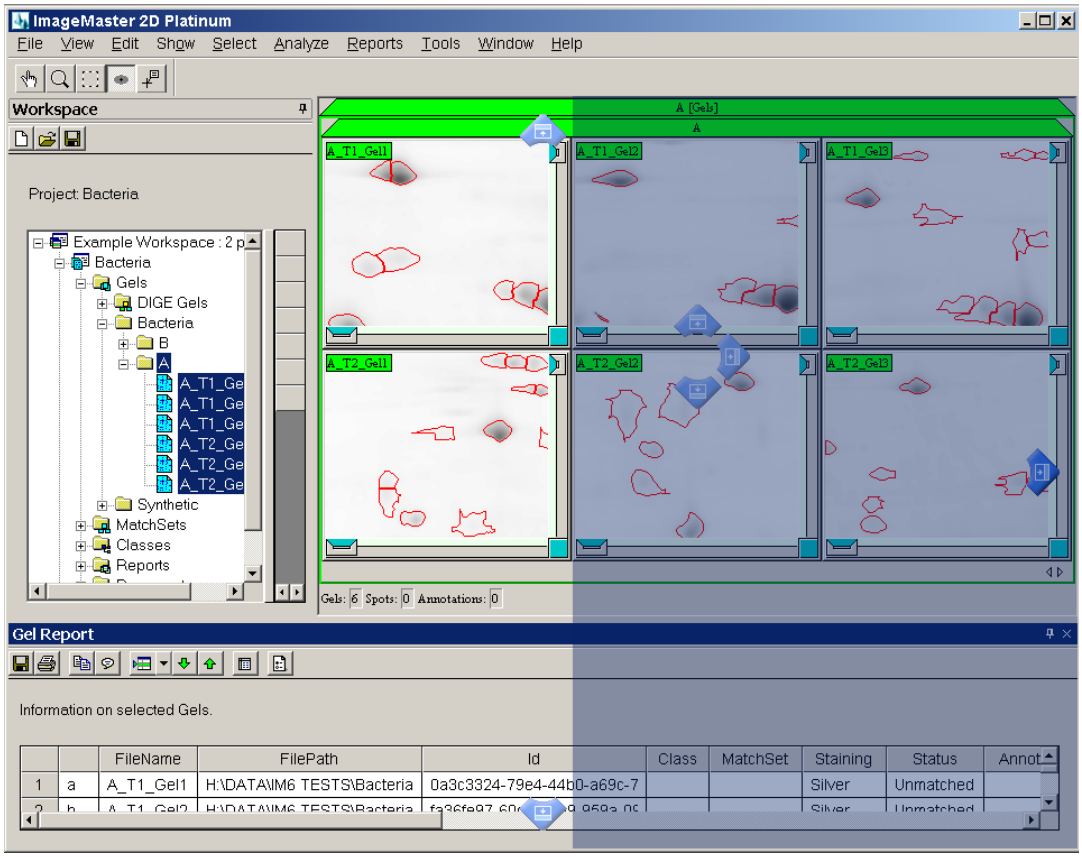


Figure 3-5. The Gel Report window previously docked at the bottom edge will now be docked at the right edge of the ImageMaster window.

Auto hide (un-pinned)

A visible window in Auto Hide (un-pinned) mode automatically collapses when not in use to become a tab on the edge of the ImageMaster window. When you click on a docked tab, the window slides back into view and is ready for use.

You can also click on Collapse to minimize a window in Auto Hide mode.

Floating

A dockable window in Floating mode will always appear on top. It can be dragged to any position within the program or even outside the main program window.

You can switch in and out of floating mode by double clicking on the title bar of a dockable window.

Tabbed groups
Dockable windows can be organized into tabbed groups. This feature extends your ability to maximize the use of limited screen space by combining multiple dockable windows into one window. In order to form a tabbed group, drag the title bar of a dockable window into the center of another. You will see the nested tabs at the bottom of the docked window. In order to separate a tabbed group, drag a tab away from the docked window or double click on the tab.

The workspace cannot be added to a tabbed group. In addition, tabular reports and graphical reports cannot be grouped together.

3.5 ImageMaster options

You can set various parameters that influence your work in ImageMaster. These settings are accessed by choosing *Tools* > *Options* in the menu. More detailed information about the options are provided in the related chapters of this manual. However, an overview of the settings, per tab, is given below.

General
- **Raw Image**: Indicates whether the raw image data of newly opened gels should be kept in memory. If the available RAM on your computer is low, then it is better not to use this option. See Section 6.4.1 for details on loading raw image data.

Cursor information
- Set the attributes to be shown in the Cursor Information window (see Section 7.9).

Display
- Indicate the default spot colors (for normal and selected spots) and spot shapes to be used (Crossed, Outlined, Filled, Outlined/Filled). Choose the color for overlapped spots and match vectors in an image.

Grid settings
- **Type**: Specify whether the grid graduations should just be regular subdivisions of the visible space - in Fixed mode - or a subdivision in terms of real coordinates at whole multiples - in Adaptive mode (see Section 6.3.5).

- **Units**: Choose the default grid units to be used for displaying a grid over a gel. Note that you can still set specific grid properties for each gel (see Section 6.3.5).

- **Divisions Number**: Specify the default number of grid subdivisions to be displayed in the horizontal and vertical directions (see Section 6.3.5).

Categories

- Set the constraints for the label categories (see Section 8.3.1) and define the color for the labels of each category.

Gel descriptions

- Add or delete gel descriptions and change their constraints (see Section 6.7.2).

Quantification

- **Volume quantification**: Indicate if you wish to compute the spot areas in mm2, based on gel resolution (default for new workspaces), or pixels (default for workspaces containing imported ImageMaster 2D Elite experiments).

Database

- **Annotations folder**: Select the folder where all linked files should be located. These files are linked to spots or pixels in a gel via file link labels (see Section 8.3.3).

- **Database**: ImageMaster offers full support for the Bruker Proteineer suite. In this context, the Server Name, User Name and Password field are used for exporting gel data to the Bruker ProteinScape database. If the Bruker tools are not installed, these parameters are inactive (grayed out).

4 Workspace

4.1 Introduction

The Workspace is the command center of ImageMaster. It can be seen as the place where all gel, matching, and analysis data is centralized and from where all operations carried out in the software are controlled.

It is where you perform the following actions:

- Manage gels, match sets, classes, reports and documents.
- Import new gels and directly convert them into ImageMaster format.
- Open gels, for viewing and processing.
- Define match sets and reference gels / match sets for matching.
- Create classes for statistical analysis.
- Add comments to describe and explain your experiment.
- Save all this information in the workspace file (with the extension .mws).

The workspace is a systematic and efficient way to clarify your research and avoid unnecessary work. The workspace ideally should reflect the structure and design of your research. This encourages you to think, right from the beginning, about the way the study should be constructed and about the information you would like to obtain from your analysis.

The workspace also guides you through the gel analysis workflow (Figure 4-1) as major operations such as spot detection, matching and statistical analysis are to be prepared in the workspace. To understand how this works, you should note that every project in the workspace is organized into five default folders: Gels, MatchSets , Classes, Reports and Documents:

- First, gels are imported in the **Gels** folder where possible relationships between gel images are specified (e.g. DIGE). The gels are then opened in the Display Zone and spot detection is carried out.
- Subsequently, **MatchSets** are created to specify how the gels will be matched together. Each match set is characterized by a reference gel and can belong to a higher level match set. Once match sets are defined, the corresponding gels can be opened and effectively matched.
- Sets of gels that will be compared are grouped in **Classes** . This is where the biological question is stated. Classes must be defined in order to

proceed with statistical analysis and the discovery of protein variations, differences and similarities in a series of gels.

- Saved ImageMaster reports are inserted in the **Reports** folder. The objects of interest in these reports can be sorted, selected, visualized on the gels and exported in different formats.
- Documents related to the project can be inserted in the **Documents** folder. Graphical files containing exported gel images, histograms, 3D views, or scatter plots are examples of files that can be added to the Documents folder. So are spreadsheet files, text files, Microsoft Word, Microsoft PowerPoint files, and many more.

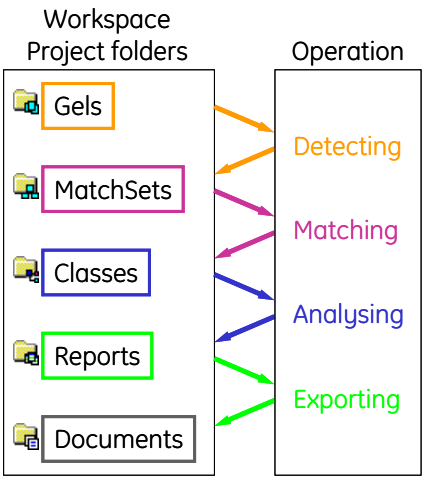


Figure 4-1. Image analysis from start to finish in the Workspace.

The gels in the workspace have icons that indicate what stage in the analysis procedure your image has reached (Table 4-1 , Table 4-2). You can distinguish DIGE gels from non-DIGE gels, see which gels have been detected and matched, and tell which gels are used as reference for matching.

Icon	Meaning
	Non-detected gel.
	Detected gel.
	Matched gel / reference gel.

Table 4-1. The gel icons indicate what stage in the analysis procedure your image has reached. Non-DIGE gels.

Icon	Meaning
	Non-detected DIGE image / DIGE reference image (e.g. standard, pool).
	Detected DIGE image / DIGE reference image (e.g standard, pool).
	Matched DIGE image / DIGE reference image (e.g. standard, pool).

Table 4-2. The gel icons indicate what stage in the analysis procedure your image has reached. DIGE gels.

The beauty of the ImageMaster workspace is that you can customize it according to your own requirements. Coworkers can look or work with the same data but organize their workspace differently.

The Workspace is essentially symbolic. Changes to the workspace do not affect images, reports, matches and master gels that are saved on your hard disk. However, the information in the workspace project is necessary to reconstruct the match sets and retrieve the matches. This is the reason why it is important to regularly save the workspace (saves the projects) and why the workspace is saved by default when closing the software.

4.2 Workspace window

Click the *Workspace*

tab below the ImageMaster toolbar to display the *Workspace* window. The window (Figure 4-2) contains the Workspace **Toolbar**, the **Active Project**, the **Navigator** and **File Details**.

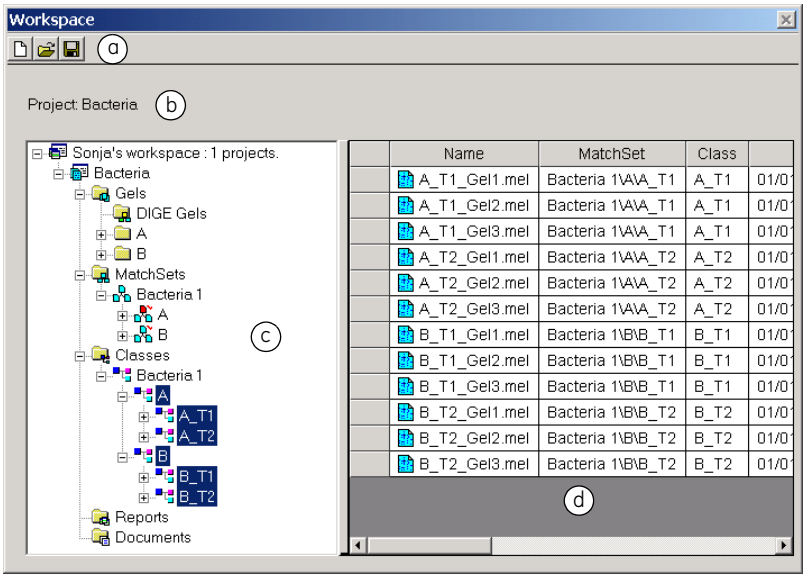


Figure 4-2. Workspace window. a) Toolbar, b) Active Project, c) Navigator and d) File Details.

4.2.1 Workspace toolbar

The following icons are available from the Workspace toolbar:

	Create a **New** workspace. In the *Create New Workspace* box, specify the Workspace Name, Location and Comment. By default, a workspace file (with the extension .mws) is saved in the *ImageMaster\Workspaces* folder found in the user's *My Documents* directory.
	Open an existing workspace. In the *Open Workspace* box, browse the directory where the workspace file is located, select its name and click *Open*.
	Automatically **Save** the current workspace including changes made to any projects. When you close ImageMaster, the workspace and projects are also saved. Keep in mind that modifications done to images, reports or matches are not saved with the workspace. To save these changes, choose one of the options under *File* > *Save*.

4.2.2 Active project

The Active Project displays the name of the project from which the active worksheet has been opened. This is particularly useful when different projects are contained in the workspace and worksheets have been opened from several of these.

4.2.3 Navigator

The Navigator displays all files and folders related to a workspace. The look and feel is similar to the Windows Explorer file manager. There is a hierarchical structure of folders, subfolders and files that can be expanded or collapsed, dragged and dropped in a new location, copied and pasted, etc.

NOTE! The main difference from Windows Explorer is that the Navigator only gives a symbolic view of your gel images, reports and inserted documents. This means that you can delete images, reports and documents from a workspace without losing the data stored on the hard disk. However, keep in mind that removing gels from a project may affect your match sets.

The fundamental organization of the Workspace is fixed. At the top of the Navigator is the Workspace Name. A workspace contains any number of **Projects**, each of which represents a study of related gels. Every project is organized into **Gels**, **MatchSets**, **Classes**, **Reports** and **Documents**. These default folders cannot be deleted or renamed. However, you can organize the Workspace according to your needs by adding subfolders to the hierarchical structure.

Workspace contextual menus

Right click on a folder, subfolder or file to open a contextual menu from which you choose an action to be carried out. Detailed descriptions of all the contextual menus in the Workspace are provided in Appendix A.

Moving files or folders

Move folders, subfolders and files in the Workspace by dragging and dropping or using the *Copy* and *Paste* options in the contextual menus.

4.2.4 File details

Detailed information about selected files is given on the right side of the Workspace window. The details include the Name, MatchSet and Class to which the image belongs, dates Created and last Modified, Size (in bytes), File Type, File Path and ID. The files can be listed in ascending or descending order by clicking on the column header that you want to sort by.

4.3 Setting up a workspace

In ImageMaster 2D Platinum 6.0, gel images can only be opened from the workspace. It is therefore necessary to set up a workspace as soon as you start working with the software.

4.3.1 Creating a workspace

To create a workspace:

1 Click on the *New* icon in the Workspace toolbar.

2 In the *Create New Workspace* box, specify the **Workspace Name**, **Location** and **Comment**. Click *OK*. By default, a workspace file (with the extension .mws) is saved in the *ImageMaster\Workspaces* folder found in the user's *My Documents* directory. If you want to save the file in a different folder, then browse and open the folder.

3 The Workspace Name appears at the top of the Navigator. The workspace file is automatically saved.

4.3.2 Opening an existing workspace

To open an existing workspace:

1 Click on the *Open* icon in the Workspace toolbar.

2 In the *Open Workspace* box, browse the directory where the workspace file is located, select its name and click *Open*.

3 The file is opened in the Workspace window.

You can also go to *File* > *Recent* > *Workspaces* in the menu and choose the Workspace Name from the list. This is only possible if a workspace file was saved during the current work session.

If you open a workspace created with an earlier version of ImageMaster, you will be asked if you want to convert it. If you click *Yes*, ImageMaster migrates the workspace so that you can use the new features in ImageMaster 2D Platinum 6.0.

NOTE! Once you open and save a workspace in ImageMaster 2D Platinum 6.0, you are no longer able to work with the file in an older version of ImageMaster. Be careful not to open version 6.0 workspace files in older versions, because this can corrupt your workspace file.

4.3.3 Workspace properties

The Workspace Name, Creator, long File Name, Modification Date and Comment are Workspace Properties. The Comment is helpful because it describes and/or explains your workspace. It serves as a useful source of information to which a coworker is referred or as a reminder when reviewing old work. You can add or modify the Comment (or Workspace Name) while viewing the Workspace Properties.

To edit the Workspace Name or Comment:

1 Right click on the Workspace Name at the top of the Navigator.

2 Choose *Properties* from the contextual menu.

3 In the *Edit Workspace Properties* box, add or modify the **Workspace Name** or **Comment**. Click *OK*.

4 The changes to Properties are saved.

4.4 *Working with projects*

A workspace can contain different projects. A project normally includes all gels, along with related data, produced and analyzed during the course of a specific gel study. In principle, a project will include images that are visually comparable in a straightforward way. Very dissimilar gel images, due to differences in staining or tissue for example, should better end up in different projects. Images that are technically related, such as multiplexed or DIGE gel images, are an exception. Since such images come from the same gel, at least their spot positions are directly comparable.

4.4.1 Creating a project

To create a project:

1 Right click on the Workspace Name at the top of the Navigator.

2 Choose *New Project* from the contextual menu.

3 In the *Create New Project* box, specify the **Project Name**, **Location** and **Comment**. Click *OK*. By default, a project file (with the extension .prj) is saved in the *ImageMaster\Workspaces* folder found in the user's *My Documents* directory. If you want to save the file in a different folder, then browse and open the folder.

4 The Project Name and default folders appear in the Navigator. The project file is automatically saved.

A project is saved whenever you click *Save* in the Workspace toolbar. You can remove a project from the current workspace by right clicking on the Project Name and choosing *Delete* from the contextual menu. This does not delete the project file from your hard disk. Therefore the project can be inserted into another workspace.

4.4.2 Inserting an existing project

To insert an existing project:

1 Right click on the Workspace Name at the top of the Navigator.

2 Choose *Insert Project* from the contextual menu.

3 In the *Insert Project* box, browse the directory where the project file is located, select its name and click *Open*. Use the Shift or Ctrl keys to make multiple selections.

4 The Project Name and the folders, gels, reports and other documents included in the project appear in the Navigator.

4.4.3 Exporting a project

Exporting a project allows you to save a project together with copies of its gels, matches, master gels, and other documents (to which new IDs are attributed), so that you can import it into a new workspace or send it to a colleague. Please note that the exported gel file structure is based on your project organization. Exported projects can be included in any workspaces as explained above.

To export a project:

1 Right click on the project to export.

2 Choose *Export* from the contextual menu.

3 Choose a destination folder for the exported project and click *OK*.

4.4.4 Project properties

The Project Name, Creator, File Name, Modification Date and Comment are Project Properties. The Project Comment is helpful because it describes and/or explains the project. It serves as a useful source of information to which a coworker is referred or as a reminder when reviewing old work. You can add or modify the Comment (or Project Name) while viewing the Project Properties.

To edit the Project Name or Comment:

1 Right click on the Project Name in the Navigator.

2 Choose *Properties* from the contextual menu.

3 In the *Project Properties* box, add or modify the **Project Name** or **Comment**. Click *OK*.

4 The changes to Properties are saved.

4.5 *Working with gels*

The **Gels** folder manages your gel images (Figure 4-3). You can easily:

- Create folders and subfolders in the default structure.
- Import gel images with .tif, .png, .gel , .img or .scan extensions.
- Add ImageMaster 2D Platinum / Melanie format gels (version 4 or higher) with a .mel extension.
- Import and define DIGE gels.
- Open images in the Display Zone, in the active worksheet or a new one.
- See which images have already spots detected on them.

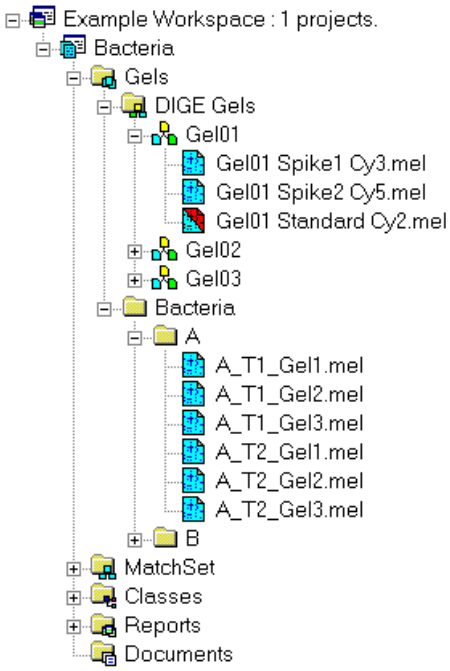


Figure 4-3. The Gels folder.

4.5.1 Creating a subfolder

You can create folders and subfolders in the *Gels* folder in order to customize the hierarchical structure and to organize your images.

DIGE gels must be placed in the designated *DIGE Gels* folder that is activated in ImageMaster 2D Platinum DIGE. Each DIGE gel holds one, two or three gel images and can be processed with the specific DIGE functionalities. The DIGE Gels folder can not contain subfolders.

To create a subfolder:

1 Right click on the *Gels* folder or a subfolder.

2 Choose *Create Folder* from the contextual menu.

3 In the *Create Folder* box, specify a **Name** and **Comment** for the new folder and click *OK*.

4 The Folder Name appears in the *Gels* folder.

Right click on the Gels folder, a subfolder or gel to open a contextual menu from which you choose an action to be carried out. Detailed descriptions of all the Gels folder menus are provided in Appendix A.

4.5.2 Importing a new gel

You can Import and automatically convert new gel images with **.tif**, **.png**, **.gel** , **.img** or **.scan** extensions into ImageMaster format (with the extension **.mel**). At this level, you can choose a reduction factor to be applied when importing the images. Thus, you automatically decrease the image resolution and therefore the file size of large gel files.

To import a new image:

1 Right click on the *Gels* folder or a subfolder.

2 Choose *Import Gels* from the contextual menu.

3 In the *Import Image* box, input the **Reduction Factor** and **File Format** and click *OK*.

4 In the *Import Gels* box, browse the directory where the image file is located, select its name and click *Open*. Use the Shift or Ctrl keys to make multiple selections.

5 In the next *Import Gels* box, specify the new names and destination folder for saving the gels in ImageMaster format. You can also add an extension to all file names, or change an existing one.

6 In the *Gel Properties* box, specify the **Staining** (the stain that was applied to the gel). Click *OK*.

7 The Gel Name appears in the *Gels* folder or subfolder that was selected in step 1. The image file is saved with the .mel extension.

4.5.3 Importing a new DIGE gel

DIGE gels must be placed in the designated DIGE Gels folder. This feature is only available in ImageMaster 2D Platinum DIGE.

To import DIGE gel images:

1 Right click on the *DIGE Gels* folder.

2 Choose *Import DIGE Gel* from the contextual menu.

3 In the *Import Image* box, specify the **Reduction Factor** and **File Format**. Click *OK*.

4 In the *Import Gels* box, browse the directory where the image files are located, select the two or three images belonging to the same DIGE gel, and click *Open*. Use the Shift or Ctrl keys to make multiple selections.

5 In the next *Import Gels* box, specify the new names and destination folder for saving the gels in ImageMaster format. You can also add an extension to all file names, or change an existing one.

6 In the *Create DIGE* box, specify a **DIGE Gel Name**, the **Dye Chemistry** (**DIGE Min.** or **DIGE Sat.**) and a **Comment**. Click *OK*. By default, the common part of the image names is suggested as gel name.

7 In the *DIGE Gel Properties* box, specify the **Staining** (the dyes that were applied to each gel). Click *OK*.

8 In the *DIGE Reference* box, specify the **Standard Gel** (typically Cy2 if using minimal dyes or Cy3 if using saturation dyes). Click *OK*.

9 The DIGE Gel Name appears in the *DIGE Gels* folder. The DIGE Gel Image Names are found below. The gel image files are saved with the .mel extension.

4.5.4 Adding an existing gel

You can add images that are already in ImageMaster 2D Platinum / Melanie format (version 4 or higher) to the Gels folder of a project. The Gels folder can only enclose one copy of each gel file.

To add an existing image:

1 Right click on the *Gels* folder or a subfolder.

2 Choose *Add Gels* from the contextual menu.

3 In the *Add Gels* box, browse the directory where the image file is located, select its name and click *Open*. Use the Shift or Ctrl keys to make multiple selections.

4 In the *Gel Properties* box, verify the **Gel Name** and **Staining** (the stain that was applied to the gel). Click *OK*.

5 The Gel Name appears in the *Gels* folder or subfolder.

4.5.5 Adding an existing DIGE gel

You can add DIGE gels that are already in ImageMaster 2D Platinum format to the DIGE Gels folder of a project. This feature is only available in ImageMaster 2D Platinum DIGE.

To add an existing DIGE gel:

1 Right click on the *DIGE Gels* folder.

2 Choose *Add DIGE Gel* from the contextual menu.

3 In the *Add Gels* box, browse the directory where the image files are located, select the 1, 2 or 3 images belonging to the same DIGE gel and click *Open*. Use the Shift or Ctrl keys to make multiple selections.

4 In the *Create DIGE* box, specify the **DIGE Gel Name**, the **Dye Chemistry** (**DIGE Min.** or **DIGE Sat.**) and a **Comment**. Click *OK*.

5 In the *DIGE Gel Properties* box, verify the **Gel Image Names** and **Staining** (the dyes that were applied to each gel). Click *OK*.

6 In the *DIGE Reference* box, specify the **Standard Gel** (typically **Cy2** if using minimal dyes or **Cy3** if using saturation dyes). Click *OK*.

7 The DIGE Gel Name appears in the *DIGE Gels* folder. The DIGE Gel Image Names are found below.

4.5.6 Moving gels or folders

You can rearrange your gels or subfolders in the Gels folder, to change their position in the list or move them into another folder. Just drag your gel or folder to the desired position. It is inserted after the item you drop it on. Whenever there is a possibility to insert it inside or after a folder, you will be asked for your choice.

4.5.7 Displaying gel images

To view gel images or work on them, they must be opened from the workspace. You can select and open individual gels, or an entire gel folder. Gels that are in

subfolders will be opened in separate panes carrying the names of the subfolders. Each DIGE gel (being the two or three images) is also displayed in a separate pane.

To open a gel or gel folder:

1 Select one or more gels or folders. Use the Shift or Ctrl keys to make multiple selections. Right click on one of the gels or folders.

2 Choose *Open* > *In Worksheet* from the contextual menu to open the gels in the active worksheet (or *Open* > *In New Worksheet* to open them in a new worksheet).

3 The selected images are opened in the active worksheet (or a new worksheet) in the Display Zone.

4.5.8 Spot detection

Once gels are added to the workspace and opened in the Display Zone, you are ready to detect spots on them. See Chapter 7 to learn more about working with spots and carrying out spot detection. Note that the gel image icons change when spots have been detected.

4.5.9 Folder properties

The Name and Comment are properties of subfolders of the Gels folder. The Comment describes and/or explains the content of the folder. It serves as a useful source of information to which a coworker is referred or as a reminder when reviewing old work. You can add or modify the Comment (or Name) while viewing the Properties of a folder. Note that you cannot modify the name of a subfolder while its gels are opened.

To edit the Comment or Name of a subfolder:

1 Right click on a subfolder of the *Gels* folder.

2 Choose *Properties* from the contextual menu.

3 In the *Gel Folder Properties* box, add or modify the **Name** or **Comment**. Click *OK*.

4 The changes to Properties are saved.

4.5.10 Gel properties

The Gel Name and Staining are Properties of any gel image file. You can modify the Staining while viewing the Gel Properties.

To edit the Staining:

1 Right click on the image name.

2 Choose *Properties* from the contextual menu.

3 In the *Gel Properties* box, modify the **Staining**. Click *OK*.

4 The changes to Properties are saved.

The Name, Dye Chemistry and Comment are Properties of DIGE gels. They can similarly be edited by right-clicking on a DIGE gel (not the individual images) and choosing *Properties* from the contextual menu.

4.6 Working with match sets

The **MatchSets** folder manages your match structures (Figure 4-4). You can easily:

- Create an unlimited number of match sets and submatch sets, enabling you to match individual gels or gel populations.
- Define a reference gel or reference match set that serves as the Master for the matching.
- Open a match set in a new worksheet, to carry out matching.
- See which gels or match sets have already been matched.
- Use your match sets to create classes.

A match set includes gels or populations of gels (in submatch sets) that should be matched together. This is a major innovation over previous versions of the software. You are no longer limited to matching gels. You can now match populations of gels, and what more is, different levels of populations. This is called population matching.

The concepts of match sets and population matching can best be illustrated with an example. Imagine a set of samples from bacteria that were cultivated with either substrate A or with substrate B. In both growing conditions, two treatments have been tested. Therefore, 4 different populations exist. A gel was run for each of the 3 samples in a population, giving a total of 12 gel images.

Different possibilities exist to construct your match sets. You could first match the three gels of each population (Figure 4-5), thus giving the match sets (i.e. populations) A_T1, A_T2, B_T1, B_T2. You could then match the populations A_T1 and A_T2 to give population A, and do the same to construct population B. Finally, the match sets A and B can be matched to give match set Bacteria 1.

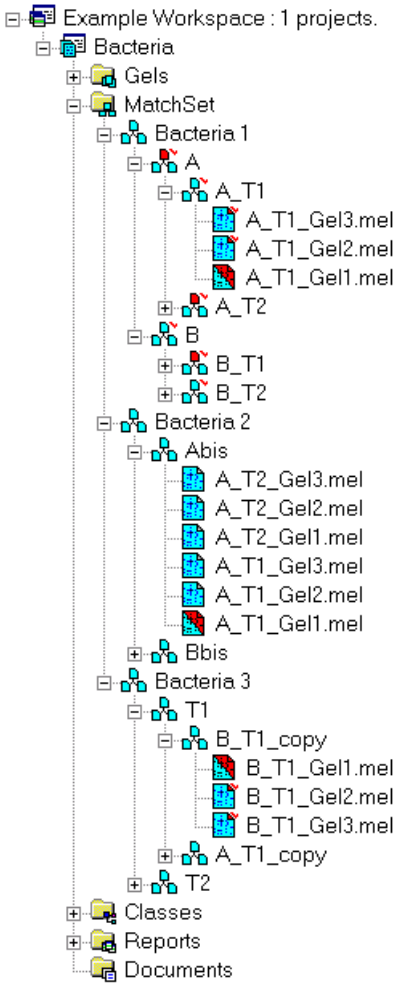


Figure 4-4. The MatchSets folder.

At each level, the matches are propagated. This means that once all match sets have been effectively matched (indicated by a red tick mark), the spots of gel A_T1_Gel1, for instance, can be directly compared with those of Gel B_T2_Gel3.

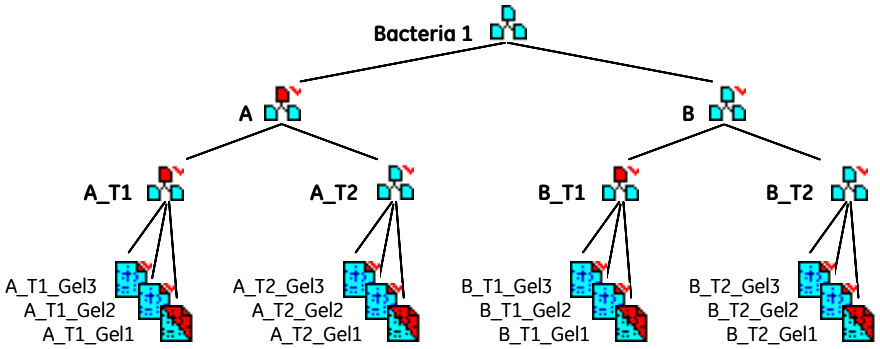


Figure 4-5. Example of a hierarchical match set structure. Case 1.

Alternatively you could directly put all gels of population A in a match set, and create a similar match set for the gels in population B. Populations A and B can subsequently be matched together (Figure 4-6).

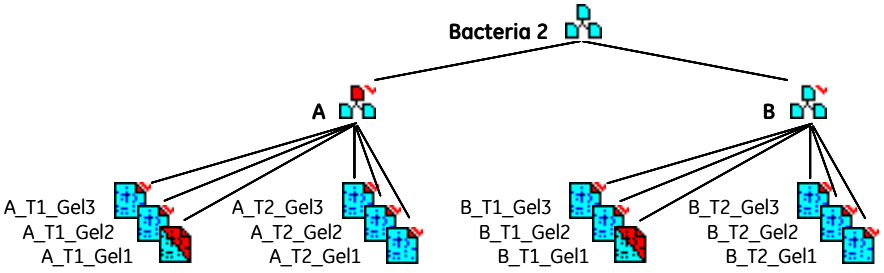


Figure 4-6. Example of a hierarchical match set structure. Case 2.

It is not possible to recommend an ideal matching scheme. Depending on how you carried out your experiment, what you would like to show in your study, or how similar your gels are, you may want to opt for a particular match set structure. You may also desire to carry out several analyses, using different matching schemes.

You can copy match sets to use them in another configuration. In Figure 4-7, for example, the match sets A_T1, A_T2, B_T1 and B_T2, were copied to be used in populations per treatment (T1, T2) rather than growing substrate (A, B). As existing matches are conserved upon copying match sets, this can save a lot of work.

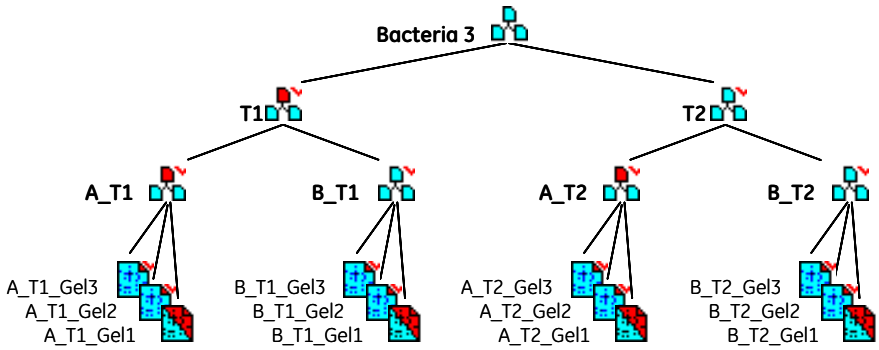


Figure 4-7. Example of a hierarchical match set structure. Case 3.

For each match set a reference gel or reference match set must be defined. This Reference will be used to create a Master, representing the match set. In Figure 4-5, Figure 4-6 and Figure 4-7, the reference gels or reference match sets have a red component.

The choice of the reference gel or reference match set is important as it significantly influences the results of your analysis. This is because spots that are not in the Reference will also not be present in the Master, unless they are manually copied there. The rule of thumb is to choose the gel with the most and the best quality spots as the reference gel. To learn more about the Reference, the Master, and matching, please refer to Chapter 9.

Please note that the master gel should rather be seen as a spot index. It is not possible to display quantification values for a master gel.

4.6.1 Creating a match set

There are different ways to create a match set:

To create an empty match set:

1 Right click on the *MatchSets* folder or a match set.

2 Choose *Create MatchSet* from the contextual menu.

3 In the *Create MatchSet* box, specify a **Name** and **Comment**. Click *OK*.

4 The MatchSet Name appears in the *MatchSets* folder or match set chosen in step 1.

To create a match set from a gel folder:

1 In the *Gels* folder, select one or more folders containing gels with saved spots. Use the Shift or Ctrl keys to make multiple selections.

2 Left click on one of the folders (a Hand cursor appears). Drag and drop the folders into the *MatchSets* folder or into a destination match set. Or right click on one of the gel folders and choose *Copy* from the contextual menu. Right click on the destination match set and choose *Paste* from the contextual menu.

3 In the *Create MatchSet* box, specify a **Name** and **Comment**. Click *OK*. Repeat for every copied folder.

4 New match sets appear in the *MatchSets* folder or destination match set. They contain the gels from the copied gel folders.

Right click on the MatchSets folder, a match set or gel to open a contextual menu from which you choose an action to be carried out. Detailed descriptions of all the MatchSets folder menus are provided in Appendix A.

4.6.2 Creating a match set from a DIGE gel

A DIGE gel is an inherent match set. As the different images in a DIGE gel have identical co-detected spots, ImageMaster automatically creates matches between the spots in the images. You can easily add a DIGE gel to your MatchSets folder or insert it into an already existing match set.

To create a match set from a DIGE gel:

1 Select one or more DIGE gels (not the individual images) in the *DIGE Gels* folder. Use the Shift or Ctrl keys to make multiple selections. Right click on one of the gels.

2 Choose *Create MatchSet* from the contextual menu.

3 In the *Add DIGE in MatchSet* box, select the match set you would like to add your new match sets to.

4 In the *Create MatchSet* box, specify a **Name** and **Comment** for each match set to be created. By default, the name of the DIGE gel is used. Click *OK*.

5 The new match sets appear in the *MatchSets* folder and the tick mark in the gel image icons indicates that matches exist between the images belonging to a same DIGE gel.

Alternatively, you can drag and drop, or copy and paste your DIGE gel to the MatchSets folder to create the corresponding match set.

4.6.3 Adding gels to a match set

There are different ways to add gels to a match set.

To use contextual menus to add gels to a match set:

1 Select one or more images in the *Gels* folder. Make sure spots were detected and saved. Use the Shift or Ctrl keys to make multiple selections. Right click on one of the images.

2 Choose *Add in MatchSet* from the contextual menu.

3 In the *Add Gels in MatchSet* box, type in a name if you want to place the gels in a new match set. Or choose an existing match set from the list. Click *OK*.

4 If a new match set is created, specify the reference gel in the following *Add Gels in MatchSet* box, and click *OK*.

5 The gels are added and the reference gel is tagged.

To copy and paste gels to a match set:

1 Select one or more gels in the *Gels* folder or *MatchSets* folder. Use the Shift or Ctrl keys to make multiple selections. Right click on one of the gels.

2 Choose *Copy* from the contextual menu.

3 Right click on the destination match set.

4 Choose *Paste* from the contextual menu.

5 The gels are added.

To drag and drop gels to a match set:

1 Select one or more gels in the *Gels* folder. Use the Shift or Ctrl keys to make multiple selections.

2 Left click on the gels (a Hand cursor appears).

3 Drag and drop the gels into the destination match set.

4.6.4 Copying match sets

Match sets can be copied for use in another match set configuration.

To copy a match set:

1 Select one or more match sets in the *MatchSets* folder. Use the Shift or Ctrl keys to make multiple selections.

2 Right click on one of the match sets and choose *Copy* from the contextual menu. Right click on the destination match set and choose *Paste* from the contextual menu.

3 The copied match sets appear in the designated location, with one or several characters appended to their names (match set names must be unique).

4 Right click on a copied match set.

5 Choose *Properties* from the contextual menu.

6 If necessary, change the match set **Name** in the *MatchSet Properties box*. Click *OK*. Note that you cannot modify the name of an open match set.

4.6.5 Setting the match set reference

For each match set, a reference gel or reference match set should be defined. It is used to create the master image that will represent the match set.

If your match set contains gels, right click on the gel to be used as Reference and choose *Set Gel as Reference* from the contextual menu. If your match set contains submatch sets, right click on the submatch set to be used as Reference and choose *Set MatchSet as Reference* from the contextual menu. Icons of reference gels and reference match sets have a red component.

NOTE! You can still change the Reference as long as the images in your match set have not been matched. Once they are, the reference image cannot be changed anymore.

4.6.6 Moving gels or match sets

You can rearrange your gels or match sets in the MatchSets folder, to change their position in the list or move them into another match set. Just drag your gel or match set to the desired position. It is generally inserted after the item you drop it on. Whenever there is a possibility to insert it inside or after a match set, you will be asked for your choice.

4.6.7 Displaying match sets

To match gels or match sets, they must be opened in a [MatchSet] worksheet.

When opening a match set with gels in it, all gels are displayed except for the Reference. The Master of the match set, recognizable through its red legend, is shown instead. This is because the master and reference images are the same, and matches are automatically created between the two. The Reference therefore no longer needs to be matched. When necessary, the reference gel can be displayed by choosing *Show* > *Show Reference* from the menu.

When opening a match set with submatch sets in it, all master gels of the submatch sets will be displayed, except for the one that was chosen as a reference for the opened match set. Instead the master image of the opened match set is shown.

To open a match set:

1 Select the match set to be opened. Right click on its name.

2 Choose *Open* from the contextual menu to open the match set.

3 The match set is opened in a new [MatchSet] worksheet in the Display Zone.

4.6.8 Matching

Once a match set is opened in the Display Zone, you are ready to match the gels against the Master. See Chapter 9 to learn more about matching. Note that the gel or match set icons change when matching is completed.

4.6.9 Match set properties

The Name and Comment are properties of match sets. The Comment describes and/or explains the content of the match set. It serves as a useful source of information to which a coworker is referred or as a reminder when reviewing old work. You can add or modify the Comment (or Name) while viewing the Properties of a match set. Note that you cannot modify the name of an open match set.

To edit the Comment or Name of a match set:

1 Right click on the match set.

2 Choose *Properties* from the contextual menu.

3 In the *MatchSet Properties* box, add or modify the **Name** or **Comment**. Click *OK*.

4 The changes to Properties are saved.

4.7 *Working with classes*

The **Classes** folder manages your classes (Figure 4-8). You can easily:

- Create an unlimited number of classes and subclasses.
- Open classes in a worksheet, to carry out statistical analysis.

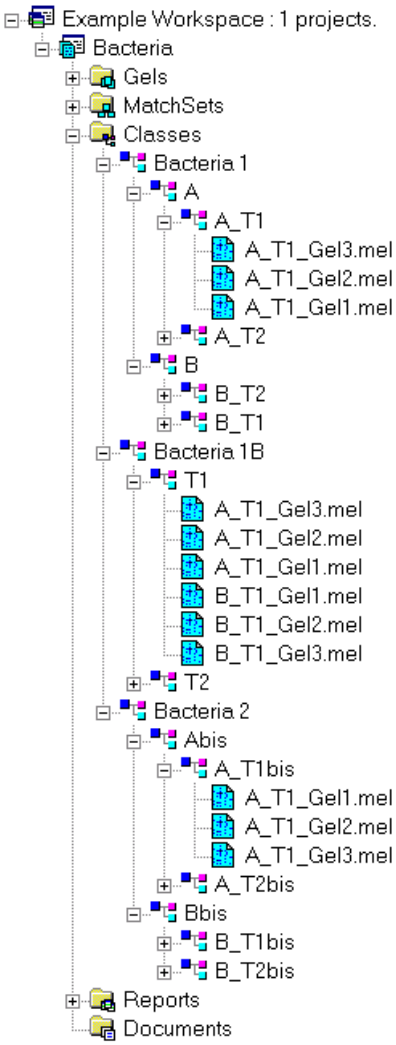


Figure 4-8. The Classes folder.

A class is a set of gels or gel populations that you can compare with other such entities. In the Classes folder, you state your biological questions. Your goal, by comparing classes and therefore the gels within those classes, is to find the protein expression variations between different biological states.

NOTE! To compare gels in a class or within different classes, the gels should have a common node in the match set that is used to construct the classes. Without a common node, the gels cannot be matched together and statistical comparison is impossible. This means that all gels to be compared should belong to the same higher level match set.

NOTE! The same series of gels may be found in several match sets. Therefore, several possibilities will exist when adding gels to a class. The decision of which match set to use can have significant impact on the statistical analysis.

The use of classes can again be illustrated with the example of the bacteria gels described in the previous section (see Section 4.6). Figure 4-8 illustrates three possible class setups based on the match set configurations Bacteria 1 (Figure 4-5) and Bacteria 2 (Figure 4-6).

The hierarchical structure of the class Bacteria 1 in Figure 4-8 is based on the match set Bacteria 1 and uses the same configuration. The class Bacteria 1 contains two sub classes A and B, which in turn have two sub classes each. Using this configuration you can compare at any moment: class A with class B, A_T1 with A_T2, A_T1 with B_T1 or even A with B_T2.

Still using match set Bacteria 1 as a basis, you can also create a class structure as in Bacteria 1B (Figure 4-8). This class contains two sub classes T1 and T2 that each hold the gels corresponding to a specific treatment. It essentially allows you to compare T1 and T2.

Another option is to use match set Bacteria 2 when defining your classes, but with the same class configuration as in class Bacteria 1. In this way, you can easily use the classes A and B, and the subclasses A_T1, A_T2, B_T1 and B_T2 for various comparisons. However, the results can be more or less different from the first case discussed above, where the Bacteria 1 match structure serves in the analysis.

NOTE! To see from which match set a gel in a class is derived, you can look at the MatchSet column in the File Details at the right side of the workspace window (Figure 4-9). It is also there that you can verify if gels belong to the same match set (necessary for statistical comparisons).

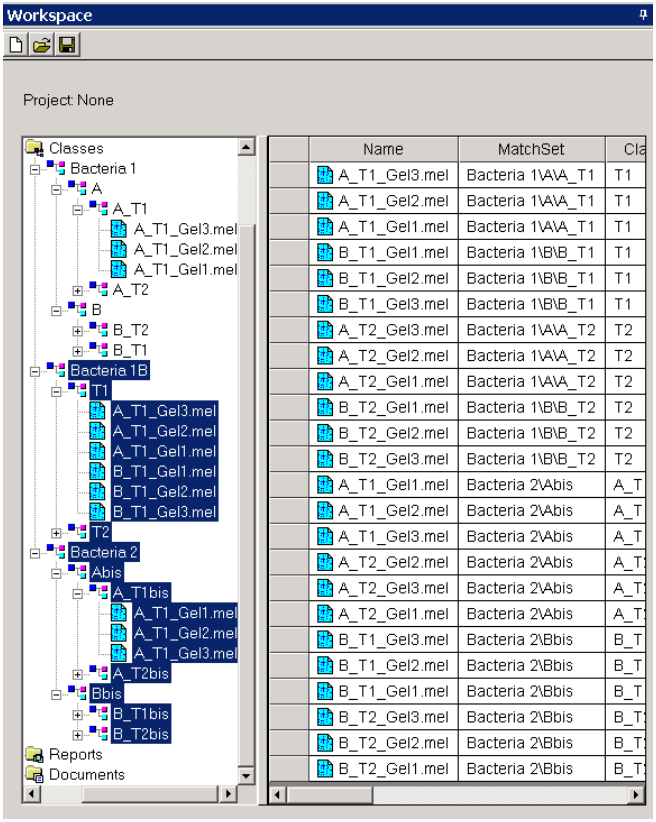


Figure 4-9. Selected gels in the Classes folder.

4.7.1 Creating a class

There are different ways to create a class:

To create an empty class:

1 Right click on the *Classes* folder.

2 Choose *Create Class* from the contextual menu.

3 In the *Create Class* box, specify a **Name** and **Comment**. Click *OK*.

4 The Class Name appears in the *Classes* folder.

To create a class from a match set:

1 Select one or more match sets from the *MatchSets* folder. Use the Shift or Ctrl keys to make multiple selections.

2 Left click on one of the match sets (a Hand cursor appears). Drag and drop the folders into the *Classes* folder or into a destination class. Or right click on one of the match sets and choose *Copy* from the contextual menu. Right click on the destination class and choose *Paste* from the contextual menu.

3 In the *Create Class* box, specify a **Name** and **Comment**. Click *OK*. Repeat for every copied match set.

4 New classes appear in the *Classes* folder or destination class. They contain the gels from the copied match set.

Right click on a class, subclass or gel to open a contextual menu from which you choose an action to be carried out. Detailed descriptions of all the Classes folder menus are provided in Appendix A.

4.7.2 Adding gels to a class

There are different ways to add matched gels to a class:

To use contextual menus to add matched gels to a class:

1 Select one or more gel images in a match set. Use the Shift or Ctrl keys to make multiple selections. Right click on one of the images.

2 Choose *Add in Class* from the contextual menu.

3 In the *Add Gels in Class* box, type in a name if you want to place the gels in a new class. Or choose an existing class from the list. Click *OK*.

4 The gels are added to the class.

To copy and paste gels to a class:

1 Select one or more gels in a match set. Use the Shift or Ctrl keys to make multiple selections. Right click on one of the gels.

2 Choose *Copy* from the contextual menu.

3 Right click on the destination class.

4 Choose *Paste* from the contextual menu.

5 The images are added to the class.

To drag and drop gels to a class:

1 Select one or more gels in a match set. Use the Shift or Ctrl keys to make multiple selections.

2 Left click on the gels (a Hand cursor appears).

3 Drag and drop the gels into the destination class.

4 The gels are added to the class.

4.7.3 Moving gels or classes

You can rearrange your gels or classes in the Classes folder, to change their position in the list or move them into another class. Just drag your gel or class to the desired position. It is generally inserted after the item you drop it on. Whenever there is a possibility to insert it inside or after a class, you will be asked for your choice.

4.7.4 Displaying classes

To carry out statistical analysis on the gels in your classes, they must be opened in a [Classes] worksheet. You can select several classes from the workspace and open them simultaneously. Each selected class will be opened in a separate pane carrying the name of the class. Note that the gels will be opened as part of the highest level class selected. This means that if you select the classes A (with subclasses A_T1 and A_T2) and B (with subclasses B_T1 and B_T2) the gels will be opened in two panes, as part of the classes A and B. If you select the four subclasses and open them, the gels will be opened in four panes, as part of the classes A_T1, A_T2, B_T1 and B_T2.

To open a class:

1 Select one or more classes. Use the Shift or Ctrl keys to make multiple selections. Right click on one of the classes.

2 Choose *Open* from the contextual menu to open the classes.

3 Each class is opened in a separate pane in the newly created [Classes] worksheet.

4.7.5 Statistical analysis

Once classes are opened in the Display Zone, you are ready to analyse your gels and calculate Intra-Class or Inter-Class statistics. See Chapter 10 to learn more about data analysis.

4.7.6 Class properties

The Name and Comment are properties of classes. The Comment describes and/or explains the content of the class. It serves as a useful source of information to which a coworker is referred or as a reminder when reviewing old work. You can add or modify the Comment (or Name) while viewing the Properties of a class. Note that you cannot modify the name of an open class.

To edit the Comment or Name of a class:

1 Right click on the class.

2 Choose *Properties* from the contextual menu.

3 In the *Class Properties* box, add or modify the **Name** or **Comment**. Click *OK*.

4 The changes to Properties are saved.

4.8 Working with reports

The Reports folder manages all tabular report files (with the extension **.rpt**) that you produce in ImageMaster. Refer to Chapter 5 to learn more about reporting.

4.8.1 Creating a subfolder

You can create folders and subfolders in the Reports folder in order to better organize your saved reports.

To create a subfolder:

1 Right click on the *Reports* folder or a subfolder.

2 Choose *Create Folder* from the contextual menu.

3 In the *Create Folder* box, specify a **Name** and **Comment** for the new folder and click *OK*.

4 The Folder Name appears in the *Reports* folder.

Right click on the Reports folder, a subfolder or report file to open a contextual menu from which you choose an action to be carried out. Detailed descriptions of all the Reports folder menus are provided in Appendix A.

4.8.2 Adding reports

When saving a report, it is automatically inserted into the Reports folder of the Project it has been created from. However, you can also add report files from disk.

To add reports:

1 Right click on the *Reports* folder or a subfolder.

2 Choose *Add Reports* from the contextual menu.

3 In the *Add Report Files* box, browse the directory where the files are located, select the names and click *Open*. Use the Shift or Ctrl keys to make multiple selections.

4 The files are added to the *Reports* folder or subfolder.

4.8.3 Opening reports

You can open a report from the Reports folder by double clicking on it, or by selecting *Open* from the contextual menu.

4.8.4 Moving reports or folders

Move folders, subfolders and files within the Reports folder by dragging and dropping or copying and pasting.

4.9 Working with documents

The Documents folder manages all project-related files other than your gel images and tabular reports. These can include text files, spreadsheet files, Microsoft PowerPoint presentations or mass spectrometry data. The Documents folder can also contain pick lists and graphical files such as histograms, 3-D views, scatter plots, factor analysis projections, exported gel regions or gel windows (with .bmp, .tif or .png extensions). When saving a graphical file in ImageMaster, it is automatically inserted into the Documents folder of the source Project.

4.9.1 Creating a subfolder

You can create folders and subfolders in the Documents folder in order to better organize your project related files.

To create a subfolder:

1 Right click on the *Documents* folder or a subfolder.

2 Choose *Create Folder* from the contextual menu.

3 In the *Create Folder* box, specify a **Name** and **Comment** for the new folder and click *OK*.

4 The Folder Name appears in the *Documents* folder.

Right click on the Documents folder, a subfolder or file to open a contextual menu from which you choose an action to be carried out. Detailed descriptions of all the Documents folder menus are provided in Appendix A.

4.9.2 Adding files

As mentioned above, any project related file, generated by any software can be inserted in the Documents folder of the project.

To add files:

1 Right click on the *Documents* folder or a subfolder.

2 Choose *Add Files* from the contextual menu.

3 In the *Add Document Files* box, browse the directory where the files are located, select the names and click *Open*. Use the Shift or Ctrl keys to make multiple selections.

4 The files are added to the *Documents* folder or subfolder.

4.9.3 Opening documents

You can open a file from the Documents folder by double clicking on it, or by selecting *Open* from the contextual menu. The file will be opened in the associated application.

4.9.4 Moving documents or folders

Move folders, subfolders and files within the Documents folder by dragging and dropping or copying and pasting.

4.10 Gel and report identifiers

ImageMaster allocates a unique identifier (ID) to each gel and report in order to assure data consistency, allow reliable identification of gels and reports across a computer network and enable database integration.

The gel and report IDs in ImageMaster are UUIDs (Universal Unique IDentifiers), which are 128 bit numbers that are guaranteed to be unique through combinations of hardware addresses, time stamps and random seeds. These IDs allow your gels and associated reports to be uniquely recognized. This is because the gel or report ID always overrides the file name or file location. Thus, ImageMaster detects, for example, if you delete a gel and replace it with another one with the same name.

NOTE! This also means that duplicating a gel outside ImageMaster may lead to confusion, as you will end up with two gels bearing the same ID. For that reason you should always try to manipulate (delete from disk, save as, rename) your gels from within ImageMaster.

When you delete, rename or move your gels and associated reports outside ImageMaster, you will get a message at program startup indicating that the file(s) can not be found. The corresponding gels and reports will also have a red cross on them in the Workspace project. To solve this problem, ImageMaster lets you search for the files, at program startup or with the *Search* option in the Workspace contextual menus. You must indicate the folder in which the files are located. If the software still can not find a file - when it has been overwritten for instance, the only option is to delete it from the workspace and insert it again.

4.11 Archiving a workspace

It is important to realize that the files included in your workspace can be located anywhere on your hard disk or network. They are not necessarily all found in the same directory. Gel and report files are usually shared by different users on one or several network folders, whereas the workspace files, project files, and matching data are by default saved in the *ImageMaster* folder of the user's *My Documents* directory (access to this data should generally be restricted).

In order to make a backup of the entire experiment or to share the files with a person that cannot access your network, you can save your workspace or just a single project with all its respective files in a single location.

NOTE! It is not possible to open a workspace/project when you don't have access to all the necessary folders, and in particular the folder where matches and other data are stored. Therefore, if a user wants to share his workspace/project, he has to share his local *ImageMaster* folder, export his project(s) (see Section 4.4.3), or provide a backup of his project or workspace (see below).

NOTE! It is good practice to make regular backups of your work using the functionalities described below, so that you can recover your work at any time.

4.11.1 Backup / Restore workspace

With the *Backup Workspace* function, a complete workspace can be archived including the projects, gels, matches, master gels, reports and other related files. This backup is written into a single compressed file (with the extension **.bkp**) that can be restored when needed. Use the *Restore Workspace* function to later retrieve the backup.

To backup a workspace:

1 Right click on the Workspace Name at the top of the Navigator.

2 Choose *Backup* from the contextual menu.

3 In the *Backup Workspace* box, browse to the directory you want, enter a file name and click *Save*.

4 The backup file (with the extension .bkp) is archived.

To restore a workspace:

1 Right click on the current Workspace Name at the top of the Navigator.

2 Choose *Restore Workspace* from the contextual menu.

3 In the *Restore Workspace* box, browse the directory where the backup file is located, select its name and click *Open*.

4 In the *Restore Backup* box, choose one of the two options for restoring a workspace. By restoring the files using the original file paths, you return to your workspace by automatically rewriting the previous file and folder structure. By restoring the files in a single folder, the workspace is reproduced in a new directory, for which you have to enter a file name and location. Click *Restore*.

5 The workspace is restored.

4.11.2 Backup / Restore project

With the *Backup Project* function, a single project can be archived including its gels, matches, master gels, reports and other related files. This backup is written into a single compressed file (with the extension **.bkp**) that can be restored when needed. Use the *Restore Project* function to later retrieve the backup.

To backup a project:

1 Right click on the Project Name.

2 Choose *Backup* from the contextual menu.

3 In the *Backup Project* box, browse to the directory you want, enter a file name and click *Save*.

4 The backup file (with the extension .bkp) is archived.

To restore a project:

1 Right click on the current Workspace Name at the top of the Navigator.

2 Choose *Restore Project* from the contextual menu.

3 In the *Restore Project* box, browse the directory where the backup file is located, select its name and click *Open*.

4 In the *Restore Project* box, choose one of the two options for restoring a project. By restoring the files using the original file paths, you return to your project by automatically rewriting the previous file and folder structure. By restoring the files in a single folder, the project is reproduced in a new directory, for which you have to enter a file name and location. Click *Restore*.

5 The project is restored in your workspace.

5 Reports

5.1 Introduction

The reports available in ImageMaster not only provide options for displaying information on selected objects, but also are useful for sifting through data. Reports can be accessed from the *Reports* menu, the *Analyze* menu or sometimes through displayed reports. Reports are divided into two different menus for the stated reasons:

- **Reports menu**: DISPLAYS only quantitative or qualitative information on selected objects (gels, spots, labels, annotations, categories and matches).
- **Analyze menu**: COMPUTES differences and similarities between gels (allowing data analysis based on robust statistics, factor analysis, statistical tests and clustering techniques).

NOTE! Reports are not necessarily in table format. Graphical representations of data such as histograms, scatter plots and 3D views are regarded as reports as well.

You can display as many reports simultaneously as you wish. Reports are Dockable Windows (see Section 3.4.4). Since every report displayed on the screen is listed in the *Window* menu, it can easily be located by clicking on its name. In order to quickly hide all pinned report windows, you can go to *Windows* > *Minimize All*. In order to close all report windows, choose *Windows* > *Close All* in the menu.

5.1.1 Reports menu

From this menu you can display reports by:

3D View	A three-dimensional view of selected gel regions or areas around selected spots.
Gel	Summarized information about the selected gels, such as Gel ID, intensity calibration parameters, number of detected spots, number of annotations, gel resolution and size, etc.
Gel Description	User-defined properties of gels such as sample type, date of the experiment, operator name, treatment conditions, pH range, SDS gel percentage, or staining.
Gel Calibration	Summarized information about the selected gels including file name, ID, file path, calibration coefficients, type of file, creator and creation date.

Spot	Specific information about selected spots, such as Spot ID and coordinates, quantification values, attached labels, etc.
Label	Information on selected labels such as content, category, Spot ID (if the label is linked to a spot), and annotation coordinates.
Annotation	Information for selected annotations about chosen categories and their labels, together with annotation coordinates and Spot ID (if the annotation is linked to a spot).
Category	Information on all labels belonging to selected categories. The displayed information is the same as for the report on labels.
Match	A list of selected matches among a number of selected gels, in terms of corresponding Spot IDs.
Match Statistics	A list of the matching results (number of matches and percentage of matches) between the master and the selected gels.

5.1.2 Analyze menu

From this menu you can display reports and histograms by:

Intra-Class	Information about each selected match such as its Match ID, value for each spot in the match, and chosen statistical measures calculated on all spots in the match. Scatter Plots also provide information (such as slope, offset, correlation coefficient and fitting error) that compare the spot values for two gels.
Inter-Class	Central tendency, dispersion and overlapping measures for classes of gels, computed for all selected matches. Differences between the spot values in two classes can also be quantified with Statistical Tests such as the Student t test, Mann-Whitney U test and Kolmogorov-Smirnov test.

Also look at:

DIGE	Reports and histograms on DIGE gels (display information such as Volume Ratios and frequency distribution of these ratios).
Heuristic Clustering	Classifies sets of gels and highlights significant matches that may allow identification of different populations and their characterization by specific spot patterns.

Most of the ImageMaster reports share a certain number of features. One of these is the standard report toolbar that offers common functionalities such as saving,

printing, selecting objects or navigating through the gel data. A second feature is that several reports are editable, more specifically those that display annotation content and properties. In addition, most of the reports are customizable. This means that you can suppress or move columns, resize columns or rows, and sort your columns in descending or ascending order based on their values (as can be done with spreadsheet software such as Microsoft Excel). Finally, the lines in tabular reports are numbered, indicating the number of selected objects used to create the report.

5.2 Report toolbars

5.2.1 Standard tools

	Save a report. Enter the File name in the displayed window and choose the desired format in the Save as type field. Tables can be saved in the ImageMaster report format (XML file carrying the extension .rpt), in text format with tabulations separating the columns (.txt), or as a Microsoft Excel Workbook (.xls). Graphics can be saved in PNG, TIFF or BMP formats.
	Print a report. When you print graphical reports (such as scatter plots, 3D views, or histograms), the printing window with the normal printing options is displayed. When you print tabular reports, things are a bit different. The report table to be printed is first displayed in your default Internet browser. This is because the XSL stylesheet located in the *Template\Reports* folder of the ImageMaster installation directory is used to transform the XML report into an attractive table (find more details about XML and XSL in Chapter 11). You can subsequently use the print option in your browser to get a paper printout.
	Copy to Clipboard. Export your data directly into another program. You can, for example, copy tables to spreadsheet software like Excel, or graphics to programs such as Word or Adobe Photoshop. To do so, first select the desired lines in a table, or the desired graphics in a window, using the Shift or Ctrl keys. Then copy the selection to the clipboard by choosing the *Copy to Clipboard* button in the toolbar or the *Copy to Clipboard* option in the contextual menu (available by clicking the right mouse button). Paste directly into the preferred software.

5.2.2 Adding comments

	Add a **Comment** to the report. You can add comments to any report to note relevant information. Since the report comment is available at any time, it is very convenient to keep information about selection criteria and other protocols for later use, and in case other people work with the report. Comments are automatically saved in the report file (when the latter is saved).

NOTE! Notice that any comment can, but need not, be saved separately using the *Save* button in the *Comment* window. Previously saved comments, as with any text file, can subsequently be opened and reused by clicking the *Open* icon. Comments can also be printed, copied or pasted, as a whole or in part, using the corresponding icons.

5.2.3 Selecting and navigating

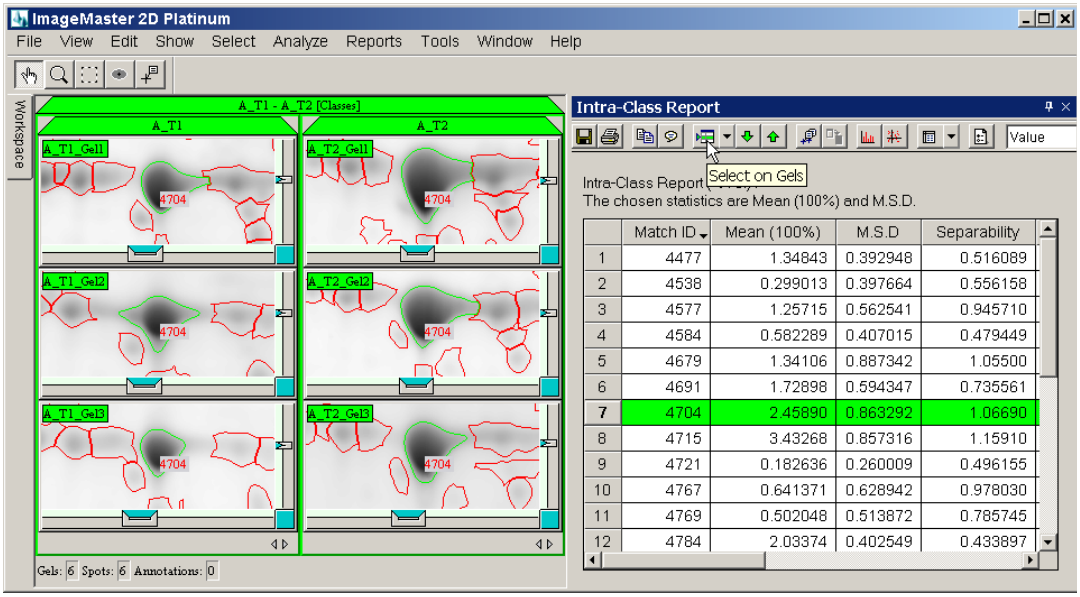
Reports have the property of being navigable, meaning that you may reselect on the gels one or more items (gels, spots, matches, etc.) that were picked in a report or histogram. You can also scroll through the items listed in a report, systematically viewing each of them on the gels by order of appearance.

Double click on a report line or histogram to select and display the corresponding object on the gel(s). If you want to select several items or go through a list of items systematically, use the following icons in the report toolbar:

	Select on Gels. When clicking on this button, any items corresponding to the selected report lines or histograms (use Shift and Ctrl keys for multiple selections) are selected on the open gels. The open gels are displaced in order to show at least one of the selected objects
	Select Next. You can use this icon to select the following object listed in the report or histogram. Please note that the open gel(s) are displaced to show the selected item in the center of the cell in which the gel is displayed.
	Select Previous. You can use this icon to select the preceding object. in the report or histogram. Again, the open gel(s) are displaced to show the selected item in the center of the cell in which the gel is displayed.

An example of how one can navigate through the lines of an Intra-class Report is shown in Figure 5-1. In order to check the matching results for particular matches, these matches were selected, and a report on matches was generated.

(a)



(b)

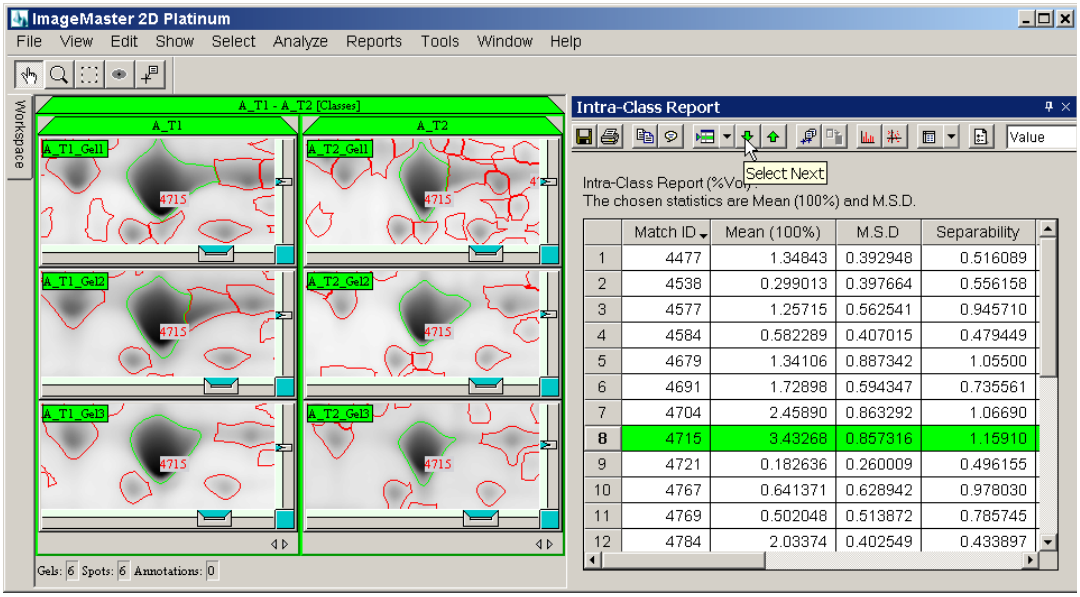


Figure 5-1. Navigating through the lines of an Intra-class Report. (a) Match 4704 is selected in the report. When the Select on Gels icon is clicked, the corresponding spots are displayed on the gels. (b) Select Next displays the following match, with ID 4715, on the gels.

After selecting the line corresponding to Match 4704, and pressing the *Select on Gels* button, all the spots belonging to Match 4704 are selected on their respective gels. By clicking the *Select Next* button, the spots belonging to the following match in the report (Match 4715) are selected on the screen. In this way, one can go through all the matches in the report. This is just a simple example to illustrate the use of reports and navigation through data. The possibilities are endless.

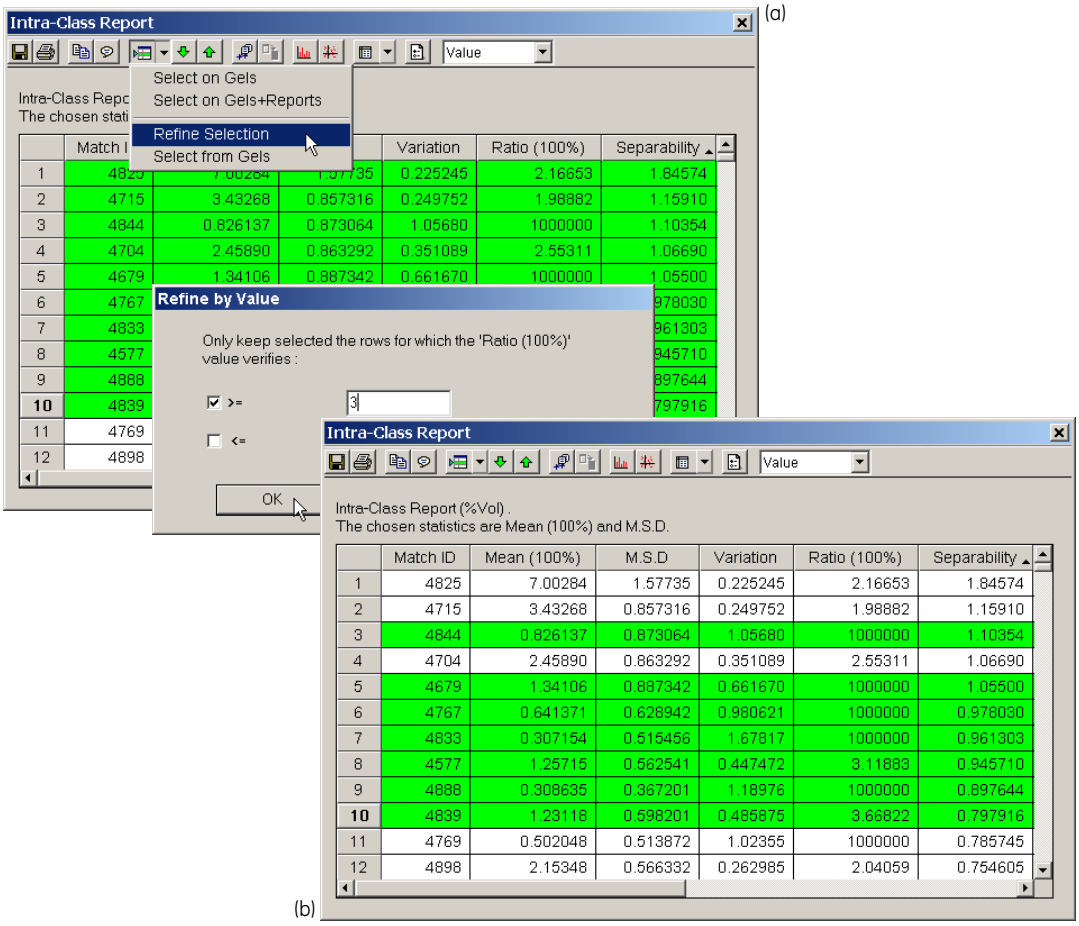
The *Select on Gels* drop down menu gives access to several related options:

- **Select on Gels** has the same function as the *Select on Gels* button.
- **Select on Gels + Reports** not only synchronizes the selection from your report to the gels, but also to any other open reports, including open histogram windows or 3D views. Imagine, for example, that you have an open Spot Report and an open Intra-Class Report. If you select a spot in your Spot Report and then choose the *Select on Gels + Reports* option, ImageMaster not only selects and displays the corresponding spot on your gel, but also selects the corresponding line in the Intra-Class Report. Of course, this is only true if your spot is part of a match, and if that match was selected for the creation of the Intra-Class Report.
- **Refine Selection** allows you to refine your selection by using an additional search criterion, applied only to the highlighted items in your table. In an Intra-Class Report, for example, you could first sort your data based on the Separability (see Section 5.4.4 for more information on sorting data), select the 10 matches with highest Separability, and then refine the selection based on the Ratio. Thus, by keeping matches that have a Ratio higher than 3, one would end up with only 7 selected matches in the example from Figure 5-2.
- **Select from Gels** will select the lines in your report that correspond to the selected objects on your gels. Please note that ImageMaster scrolls up or down in the report so that at least one selected line becomes visible.

5.2.4 Displaying related reports

Next to some report specific icons such as those linked with the *Histograms*, *Measure Histograms* or the *Factor Analysis* functions (which are discussed in the pertinent sections of this manual), you may find the following button:

	In most cases, it allows you to create a **Report from Selection**, that is, a new report that only contains the selected lines (or histograms) from the active report. Thus, you can create a new report, only containing objects that fit your specific criteria, without first having to reselect the items on the gel.



	Match ID	Mean (100%)	M.S.D	Variation	Ratio (100%)	Separability
1	4825	7.00284	1.57735	0.225245	2.16653	1.84574
2	4715	3.43268	0.857316	0.249752	1.98882	1.15910
3	4844	0.826137	0.873064	1.05680	1000000	1.10354
4	4704	2.45890	0.863292	0.351089	2.55311	1.06690
5	4679	1.34106	0.887342	0.661670	1000000	1.05500
6	4767	0.641371	0.628942	0.980621	1000000	0.978030
7	4833	0.307154	0.515456	1.67817	1000000	0.961303
8	4577	1.25715	0.562541	0.447472	3.11883	0.945710
9	4888	0.308635	0.367201	1.18976	1000000	0.897644
10	4839	1.23118	0.598201	0.485875	3.66822	0.797916
11	4769	0.502048	0.513872	1.02355	1000000	0.785745
12	4898	2.15348	0.566332	0.262985	2.04059	0.754605

Figure 5-2. Refining a selection in your report. (a) The matches in the report were sorted according to their Separability (in descending order), and the first 10 matches were selected. This selection was further refined by only keeping the rows for which the Ratio (100%) value is higher than 3. The result is shown in (b).

However, when you find a drop down menu next to the icon, the ImageMaster tool tip calls it the **Reports** icon. In this particular case, the drop down menu gives you a list with several related reports. These can include:

- **Report from Selection**: Generates a new report only containing the selected lines from the active report (as describe above).
- **Gel Report**: Contains summarized information about the gels selected for the creation of the active report and always includes the master gel, if one

has been created. It can be used as a legend to the gel index (a,b,c,...) on histograms. Note that this report is not necessarily the same as the one obtained by choosing *Reports* > *Gel Report* in the menu.

- **Spot Report**: Is a modified report on spots that is helpful to compare one spot against the others in the same match. The usefulness of this report is described in more detail in the Chapter 10 of this manual.
- **Intra-Class Report**: Displays a report on selected matches for a selected class. It is a kind of reduced report on matches, where only the spot values of one class are considered.
- **Inter-Class Report**: Lists the numerical values corresponding to selected histograms.
- **Fitting Report / Scatter Report**: These reports are only available when displaying scatter plots and are detailed in Chapter 10 of this manual.
- **DIGE Report**: Is a modified report on spots for DIGE gels, which allows to display Volume Ratios between two selected images of the same DIGE gel, in addition to the other quantification values for spots.

5.3 Editing report content

Some reports in ImageMaster (on matches, classes, annotations, labels, and categories) are editable, although to different extents. Within the Label and Category Reports, you can edit existing annotations on any gel used for the creation of the report. In the Intra-Class, Inter-Class and Annotation Reports, you can additionally create new columns corresponding to label categories and add new labels. However, as the annotations displayed in some of these reports (Intra-Class, Inter-Class) are those from the master gel, any modifications are only applied to the latter. This is not a problem, since you can easily propagate selected labels to matched spots (see Section 8.6.2). The ability to edit reports gives you the possibility to easily mark any interesting data from within the report and to propagate this data to your master gel, resulting in the creation of new annotations on this master gel.

Exhaustive information about annotations, categories and labels is given in Chapter 8. However, at this point it is useful to know that you can create different label categories, which often correspond to columns in your report, and that you can apply special constraints to the label content in each category. These include the **Data Type** (Text, Number or Boolean) and whether each label in the category should be **Unique** (as is the case for landmarks) or not.

	The **Annotate icon** allows you to insert a category as an extra column by selecting its name from the category list. You can create a new category by typing its name in the upper field of the displayed window. In the latter case, you are asked for the category constraints.

Once this is done, you can directly add new labels to the appropriate cells or edit existing ones. Just double click in a cell to start typing your label. When finished, a single click in any cell quits the editing mode. The cell corresponding to the modified label appears in dark green when the corresponding line is selected or in gray when it is not selected. You can quickly see that modifications were made to your report when an asterisk follows the window title.

	Subsequently, the **Update Gel** icon should be used to make the modifications definitive and to propagate the information to your gels (which will correspond to the master gel for the Intra-Class and Inter-Class Reports). Note that the cells of updated labels go back to their original color. When all labels in your report have been updated, the asterisk behind the report title also disappears.

NOTE! A word of warning is expressed here. Any changes made to a gel (addition or modification of labels) by using the *Update Gel* icon can be canceled with the undo function. This is not the case for reports. Editing operations on reports cannot be reversed.

Notice that categories of the type Set, or categories using the Boolean Data Type, are displayed in the form of check boxes in some reports. Editing these labels corresponds to checking the box in order to indicate that the item belongs to a Set (box checked) or not (empty box), or takes the value 0 (empty box) or 1 (box checked) for a Boolean Data Type.

To check several boxes (i.e. lines) simultaneously:

1 Select the rows you want to change the checked state.

2 Hold down the Shift key while clicking in the box of one of the selected rows (of course in the relevant column). All selected lines are now in the same activated state. Click in the check box a second time if the state should be reversed.

5.4 Customizing reports

5.4.1 Settings

	The presence of the **Settings** icon indicates that the current report is completely customizable. This means that you can choose the data columns to build your personalized report and the order in which they should be displayed. This is particularly useful when you only want the essential information to appear for clarity or for printing purposes.

To customize your report:

1 Click on the *Settings* icon in the report toolbar.

2 In the displayed window, select the attributes that you would like to add to the report from the left column (**Gels** or **Master gel** list, in the case of Figure 5-3), and transfer them to the right column (called **Visible attributes** list, in the case of Figure 5-3) by pressing the *right-pointing arrow* button.

3 To hide columns in the report, select their names in the right column and transfer the chosen items to the hidden list(s) at the left by pressing the *left-pointing arrow* button.

4 Click *OK* to confirm your choice and view the customized report.

Please note that the order of the items in the list of visible attributes determines the order of the columns in your report (although the column order can later be changed, as explained below). Thus, you can manipulate the column order by adding your attributes in a specific sequence to the list of visible attributes.

Your adapted settings can be saved and used as report templates. For this purpose, the following options are available from the *Settings* window:

- By clicking **Save** and typing a file name you can save the new report template.
- By clicking **Load** and choosing a file name you can load an existing template file.

There is no limit to the number of report templates that you can save or use. If you save a report template in the *ImageMaster\Menus* folder of the User Documents directory, it is available at any time from the menu *Reports* > ... *Report* (the templates are automatically loaded when ImageMaster starts). If the template is saved in or loaded from any other folder, it is added to the menu *Reports* > ... *Report* > *Recent Templates*, and is only accessible during the current work session.

The last report template used is available by choosing *Reports* > ... *Report* > *Current Template* from the menu.

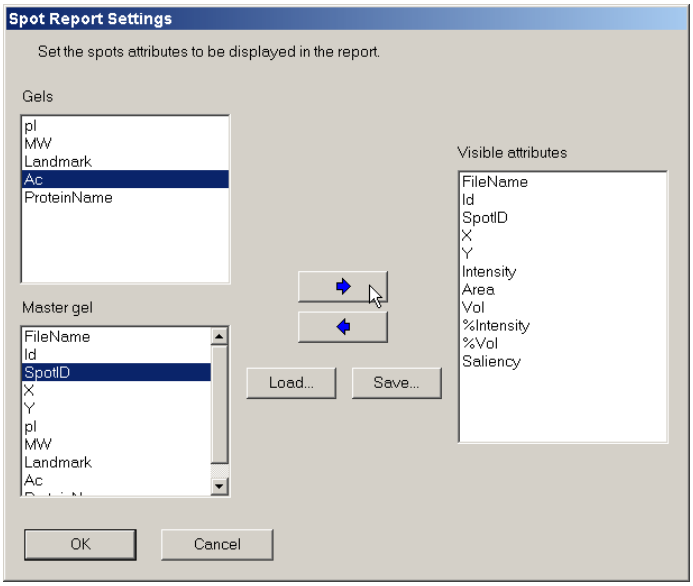


Figure 5-3. Spot Report Settings window. You can add items to, or suppress items from, the Visible attributes list using the corresponding blue arrow buttons.

5.4.2 Column order

You can reorganize the columns of a table directly in the report window. To do so, drag the column heading to its new position, that is, to another column to which it will be inserted to the left.

5.4.3 Column and row size

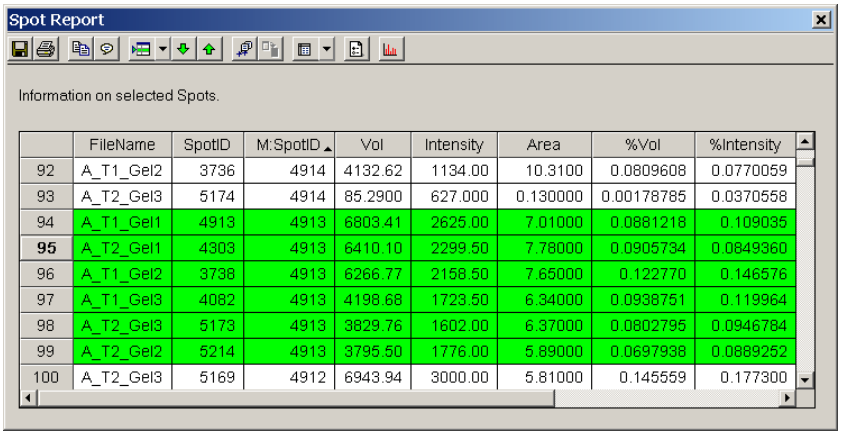
You can enlarge or reduce the column size. Drag the boundary on the right side of the column header until the column is the width you want. While dragging, you will notice that the cursor changes its format. To simultaneously change the width of all columns, hold the Shift key and then drag the boundary of any column header. To resize columns so that their whole content is displayed, double click on their separator. The column to the left of the cursor reverts to its default size.

You can also alter the row size. In this case, drag the lower border of the row to reduce or increase its height. To enlarge or reduce all rows at once, hold down the Shift key and drag the row separator. To resize rows so that their whole content is displayed, double click on their separator.

5.4.4 Sorting data

Data in tabular reports can be sorted by the column content. If you click once on the header of a specific column, a triangle is displayed indicating that the column's numerical data is sorted in descending order and the textual data in ascending order. When you click once more on the header, the triangle inverts indicating that the data is sorted in the opposite order. Note that ascending order means that numbers are sorted from 0 to 9 and text is sorted from A to Z.

You can sort the items in your table using multiple criteria. Since rows containing identical values in a sorted column appear together, you can further sort the items in your table by specifying additional columns for ranking. Note that if you would like to sort your data by two or more criteria, you should sort by the least important columns first and finish with your principal criterion. In Figure 5-4 the principal criterion is the Spot ID of the master gel (Match ID) and the secondary criterion is the spot Volume.



	FileName	SpotID	M:SpotID	Vol	Intensity	Area	%Vol	%Intensity
92	A_T1_Gel2	3736	4914	4132.62	1134.00	10.3100	0.0809608	0.0770059
93	A_T2_Gel3	5174	4914	85.2900	627.000	0.130000	0.00178785	0.0370558
94	A_T1_Gel1	4913	4913	6803.41	2625.00	7.01000	0.0881218	0.109035
95	A_T2_Gel1	4303	4913	6410.10	2299.50	7.78000	0.0905734	0.0849360
96	A_T1_Gel2	3738	4913	6266.77	2158.50	7.65000	0.122770	0.146576
97	A_T1_Gel3	4082	4913	4198.68	1723.50	6.34000	0.0938751	0.119964
98	A_T2_Gel3	5173	4913	3829.76	1602.00	6.37000	0.0802795	0.0946784
99	A_T2_Gel2	5214	4913	3795.50	1776.00	5.89000	0.0697938	0.0889252
100	A_T2_Gel3	5169	4912	6943.94	3000.00	5.81000	0.145559	0.177300

Figure 5-4. Rows in a Spot Report sorted by two criteria. Spots were first sorted according to their Volume (in descending order), then according to their Match ID.

5.5 Mouse selection reports

Mouse selection reports (Figure 5-5) are similar to any standard report in terms of functionality (icons, etc.) The main difference is that they are interactive. The reports contain only the items you select with the mouse while using the *Spot* or *Annotation* tools. You can select several items at a time, by holding down the Shift or Ctrl key, but when deselecting them they immediately disappear from the report.

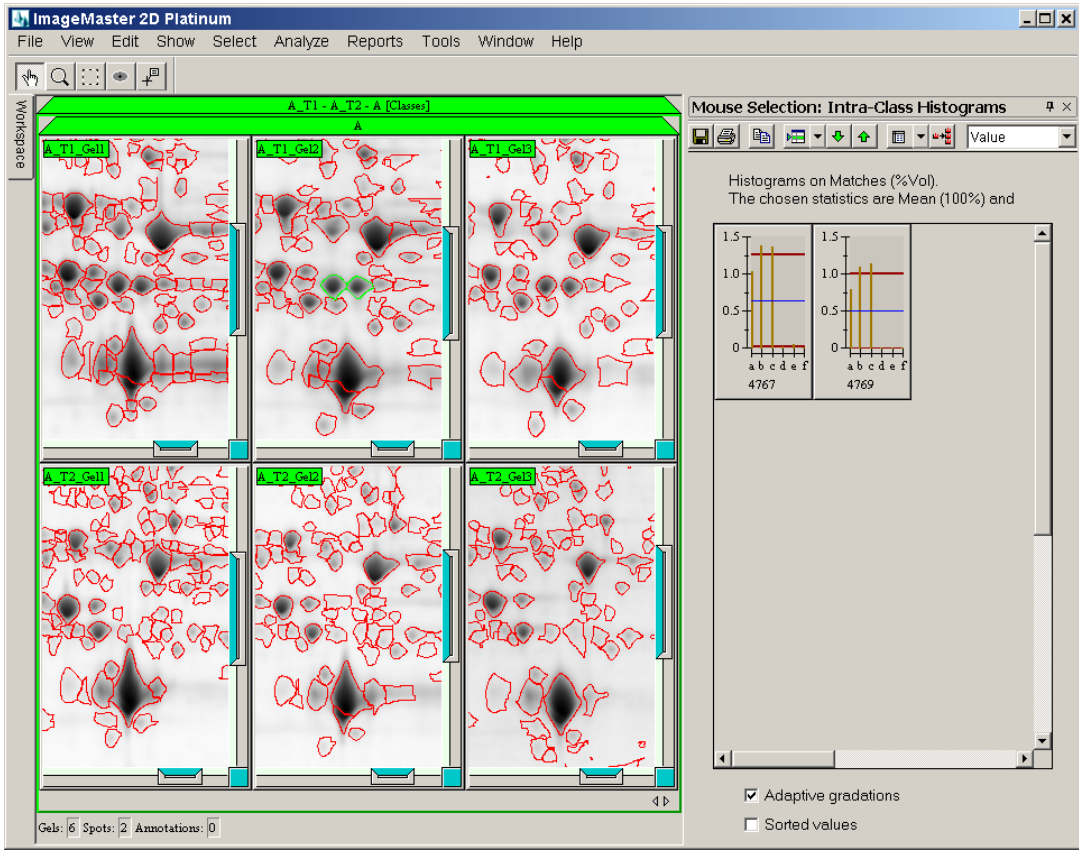


Figure 5-5. Mouse selection version of the Intra-Class Histograms. Only the histograms for currently selected objects (matches 4767 and 4769) are displayed. If the spot from match 4767 is deselected, only the histogram of match 4769 will be shown.

This type of report has the advantage of only displaying information on objects of interest. Moreover, you do not have to open a new report for each additional item.

The following report types are available from the *Window* > *Mouse Selection* menu:

- 3D View
- Spot Report
- Label Report

- Intra-Class Report
- Intra-Class Histograms
- Inter-Class Report
- Inter-Class Histograms

6 Gels

6.1 Introduction

This chapter presents ImageMaster operations that relate to gels. You will learn how to do the following:

- Change the display of gel images (zooming gels, adjusting the contrast and looking at intensity profiles or 3D views).
- Create or verify an intensity calibration.
- Process your gels (rotating, flipping, scaling, cropping).
- Save, print and export gel images.

To make any analysis meaningful, it is important to start with good quality image files. The following paragraphs give some helpful tips on what image format, resolution and depth should be used to obtain the best results.

6.1.1 Image format

Input formats

Your gels must first be digitized by an imaging device (such as a flatbed document scanner, camera system, densitometer, phosphor imager or fluorescence scanner) and then saved in an appropriate file format. The recommended formats are TIFF (Tag Image File Format), PNG (Portable Network Graphics), GEL (Molecular Dynamics), IMG (Fuji) and SCAN (Bio-Rad). It is important to remember that ImageMaster uses the calibration information contained in these image files.

ImageMaster format

After opening your gels for the first time in ImageMaster, they are saved in the ImageMaster 2D Platinum file format (unless otherwise stated). ImageMaster gel files use the extension .mel.

6.1.2 Image resolution

The scanning resolution of the gel is critical as it influences the amount of visible detail in the image. A low resolution corresponds to a large pixel size or a small number of pixels (or dots per inch). When the image resolution is too low, individual spots cannot be distinguished. On the other hand, when the scan resolution is too high, the image file becomes very large, and this slows down the gel analysis significantly. A resolution between 150 and 300 dpi is generally sufficient for gel analysis.

Please note that the 1:1 (or 100%) zoom is recommended when capturing an image with a scanner. Shrinking the image often leads to a loss of quality or the

inability to resolve data. Increasing the size of the image is useless because it does not add any information. Camera-based systems do not capture at a set scale. When using such systems, you generally end up with different resolutions in the final images. Although this is not a problem for ImageMaster, working with identical resolutions certainly facilitates image manipulation (for example, zooming or alignment).

6.1.3 Image depth

The range of potential gray levels in an image varies according to the image depth. In the case of an 8-bit image, one pixel has 256 possible gray values (0 to 255). Images scanned with a higher image depth contain more information. A 16-bit image (65536 gray levels) will reveal more subtleties. We strongly recommend an image depth of at least 12 bits for gel analysis.

When carrying out an intensity calibration, the raw pixel values are converted into real world units (generally optical density or OD). This means that the range of gray values is the same no matter what the original image depth. Some scanning devices produce images that are already calibrated. ImageMaster takes into account the conversion tables or calibration formulas stored in the files exported by such devices. If necessary, you can perform a calibration of the image capture device within ImageMaster, using calibration step wedges or calibration strips (see Section 6.5).

6.2 Selecting gels and gel regions

Most of ImageMaster's functions can only be performed on selected gels in the active worksheet. Gels are selected by clicking on their legends, using the *Select* menu options or via a report. Gel regions are defined using the *Region* tool.

Legends

Each image has a legend with its name in the upper left corner. The color of the legend indicates whether the image is selected (green, or red for a master image) or not (gray, or pink for a master image). In order to select an image, click on its legend. Use the Shift or Ctrl keys to make multiple selections.

Select menu

ImageMaster allows you to select gels based on specific criteria. Choose *Select* > *Gels* in the menu and select one of the following options:

- **All**: Selects all gels that are found in the active worksheet. The shortcut for this option is Ctrl+A.
- **Aligned**: Selects only the aligned gels.
- **Inverse selection**: Reverses the selection of gels.

Reports

When you double click on gels, spots, annotations or matches in a report or use the *Select on Gels* icon in the report toolbar, the related gels are automatically selected in the active worksheet. This means that when you double click on a spot in a Spot Report, the gel containing that spot is selected. When you double click on a match in an Intra-Class Report, all gels containing that matched spot are selected.

Region tool

A region is a rectangular area in a gel that is used for different purposes in ImageMaster. Certain actions can be limited to this region such as the selection of spots or annotations. You can use a region to preview spot detection parameters or gray level adjustments. You can also save, print or export a region as well as display a 3D view of a selected area.

To define a region in a gel:

1 Click on the *Region* tool in the toolbar.

2 Position the cursor at the top left position of the area you want to select, hold down the left mouse button, and move the cursor to the bottom right position. Release the mouse button at the end point.

3 A dashed box outlines the region (Figure 6-1).

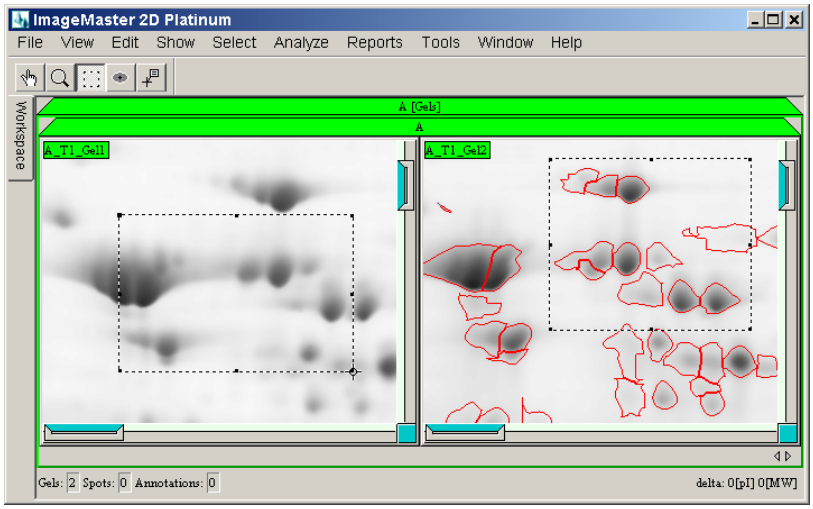


Figure 6-1. Region tool. The left gel shows a region during selection (no spots displayed), the right one after selection.

You can move a region by clicking inside the box and dragging it. You can also change the size of the box by dragging a corner or edge. In order to remove a region, select the *Region* tool (if not already done) and double click on the gel.

In order to define the same region on all gels in the active worksheet, hold down the Shift key while drawing the box on one of the gels. This region is defined in the other gels based on the corresponding pixels.

6.3 Displaying gels

6.3.1 Moving gels

There are several ways to change positions in gels. You can use the *Hand* tool, *Show* menu options, shortcut keys or scrollbars (see Section 6.3.3). You can also double click on a gel to move all gels in the active worksheet to the same position.

Hand tool
The *Hand* tool is the most straightforward means of moving gels.

To move a gel:

1 Click on the *Hand* tool in the toolbar.

2 Click on the gel and hold down the left mouse button while moving the cursor. The image changes position.

3 Release the mouse button at the position you want.

In order to move all gels in the active worksheet by the same displacement, hold down the Shift key while changing the position in one of the gels.

Double click
In order to move all gels in the active worksheet to the same position with the same magnification, select the *Hand* tool (if not already done) and double click on one of the gels. The corresponding position in the different gels is estimated by interpolating between the surrounding matches, or if such matches do not exist, between the two nearest common landmarks (i.e. landmarks with the same name). Finally, when no such landmarks exist, the gels are aligned at the same location using the X and Y coordinates.

Show menu
ImageMaster allows you to move gels based on specific positions. Choose *Show* > *Gels* > *Move* in the menu and select one of the following options:

- **Same location**: All gels in the active worksheet are moved to the same position with the same magnification as the selected gel.

- **Up**: Scroll up in the selected gels
- **Down**: Scroll down in the selected gels.
- **Left**: Scroll to the left in the selected gels.
- **Right**: Scroll to the right in the selected gels.
- **Top Left**: Display the top left of the selected gels.
- **Top Right**: Display the top right of the selected gels.
- **Bottom Left**: Display the bottom left of the selected gels.
- **Bottom Right**: Display the bottom right of the selected gels.

6.3.2 Zoom gels

There are several ways to enlarge or reduce the view of gels. You can use the *Magnify* tool, *Show* menu options, *Zoom* window, *Overview* option or scrollbars (see Section 6.3.3).

Magnify tool

To zoom in or out on a gel:

1 Click on the *Magnify* tool in the toolbar (Figure 6-2).

2 To zoom in, repeatedly click the area of the gel where you want to see details.

3 To zoom out, right click repeatedly on the gel.

To move all gels in the active worksheet to the same position with the same magnification, hold down the Shift key while zooming in or out on one of the gels.

Show menu

ImageMaster allows you to zoom in and out on gels based on specific factors. Choose *Show* > *Gels* > *Zoom* in the menu and select one of the following options:

- **In**: Zoom in on the selected gels.
- **Out**: Zoom out on the selected gels.
- **1/16**: Reduce the original image by a factor of 1/16.
- **1/8 ... 16**: Use one of the intermediary zoom factors.
- **32**: Enlarge the original image by a factor of 32.
- **Fit in Screen**: Show the full image in the cell.

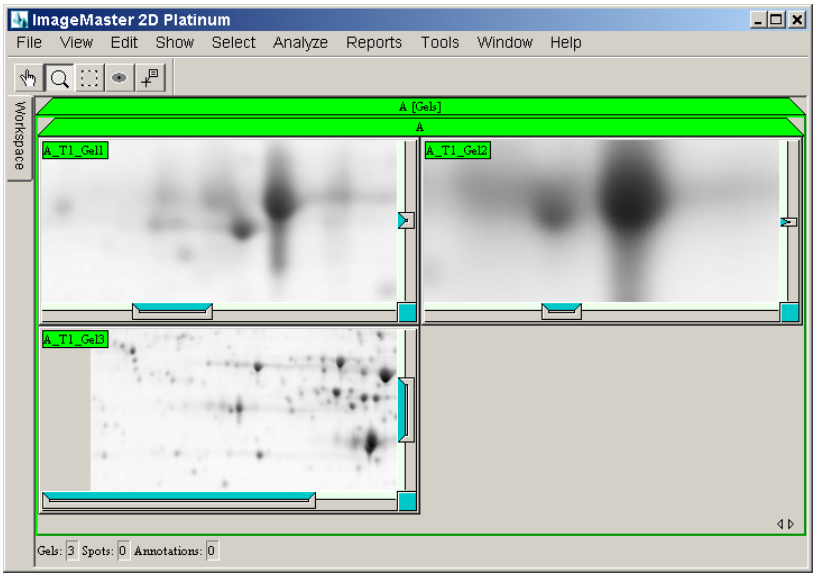


Figure 6-2. Magnify tool. The upper left gel is displayed without zoom factor. The upper right gel was zoomed 2-fold, whereas the lower gel was zoomed out by a factor of 0.25.

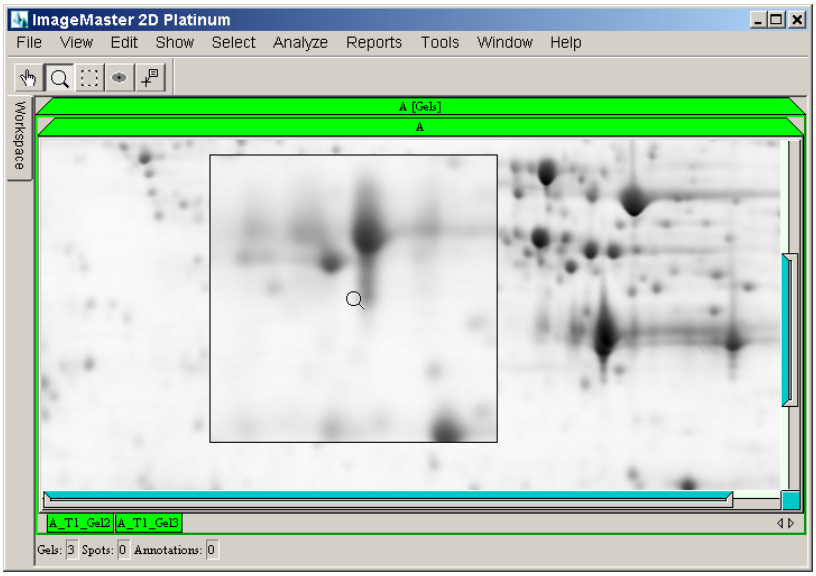


Figure 6-3. Magnifying glass.

Magnifying glass

To temporarily enlarge an area of a gel:

1 Click on the *Magnify* tool in the toolbar.

2 Hold down the Ctrl key and click on the region you want to enlarge.

3 The area under the cursor is magnified (Figure 6-3).

Zoom window

The *Zoom Window* has two functions. First, it can be used to enlarge the area of the gel under the cursor (Figure 6-4). This is the case when the zoom factor in the Zoom Window is greater than that of the gel. Second, the Zoom Window can be used to see the visible area in a larger view of the gel (Figure 6-5). This is the case when the zoom factor in the Zoom Window is less than that of the gel.

To see the enlargement of the gel area under the cursor:

1 Choose *Window* > *Zoom* in the menu.

2 In the *Zoom Window*, set the zoom factor by clicking the + and - buttons. The zoom factor must be greater than that of the gel. You can also adapt the zoom factor of the gel if needed.

3 Position the cursor on the area of the gel where you want to see details.

4 The region under the cursor is magnified in the *Zoom Window* box.

To see the visible area in a larger view of a gel:

1 Choose *Window* > *Zoom* in the menu.

2 In the *Zoom Window*, set the zoom factor by clicking the + and - buttons. The zoom factor must be less than that of the gel. You can also adapt the zoom factor of the gel if needed.

3 Position the cursor on the gel you want to view.

4 The visible area is localized in a green box in the *Zoom Window*.

The Zoom Window can be resized by dragging a corner or edge. Spots or annotations are also displayed in the Zoom Window (for zoom factors higher than or equal to 1).

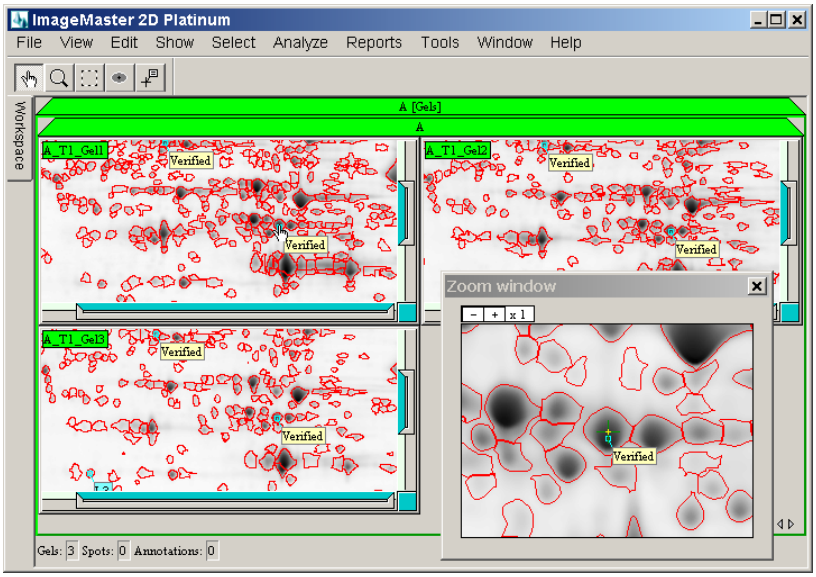


Figure 6-4. Zoom Window used to enlarge the region under the cursor. The exact cursor position is indicated by the green cross.

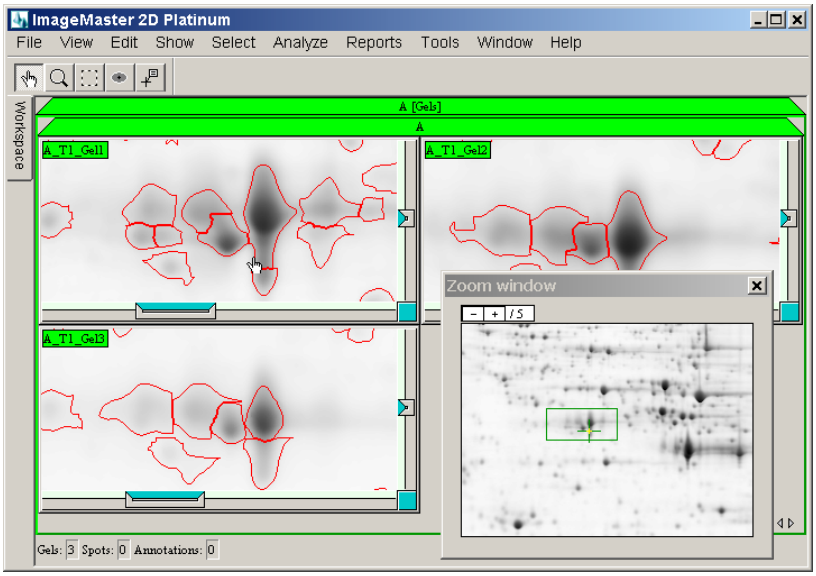


Figure 6-5. Zoom Window used to localize the visible gel area (represented by a green box) in a larger view of the gel. The exact cursor position is indicated by a green cross.

Overview option

When you zoom in on a gel, it can be helpful to have an overview of where the region is localized on the full image (Figure 6-6). This overview enables you to easily locate and move to any region you want on your gel.

To display and use the Overview option:

1 Choose *View* > *Overview* in the menu. If *Overview* is checked, then the option is activated.

2 The overviews appear in the lower right corners of all images in the Display Zone. The green rectangle in the overview corresponds to the current view of the gel.

3 Drag the green rectangle to another position to display a new region.

4 To deactivate this option, choose *View* > *Overview*. *Overview* is no longer checked in the menu. This is the default setting.

Figure 6-6. Overview option activated. In the lower right corner of all images in the Display Zone is a small overview of the entire gel with a green rectangle corresponding to the visible gel area.

6.3.3 Scrollbars

Scrollbars (in blue and grey) are given on the right and bottom edges of each image. The size of a scroll box indicates the proportional amount of the used area of the image that is visible in the cell. The position of the scroll box indicates the

relative location of the visible area within the image. These scrollbars enable you to:

- Change the zoom factor of the image. The cursor of the mouse changes to a double-sided arrow icon when placed over the respective scroll box. Click the mouse on the edge of either end of the scroll box and change the length of the box. The image zooms in or out depending upon if you make the scroll box shorter or longer. The other scroll box scales accordingly. By clicking on the blue square box at the intersection of the two scrollbars, you can reset the image to its full image size.
- Move the visible area of the gel up, down, left or right. Simply place the cursor of the mouse over the scroll bar, click the mouse and move in the direction you want.

It is possible to hide the scrollbars by deselecting *View* > *Scrollbars* > *Show* in the menu.

If you want to view a specific area of your gel image, choose *View* > *Scrollbars* > *Adjust*. In the *Adjust Visible Area* window, set the exact horizontal and vertical start and end coordinates of the area to be displayed. You can do this in terms of different units: **Image coordinate**, **Percentage** and **Real value** (pI and molecular weight). Please note that pI_MW annotations have to be defined in order to use the Real value option.

6.3.4 Transparency

The *Transparency* mode is used to visualize similarities or differences between the current gels and the specified reference gel. Two options exist:

- **Image transparency**
- **Spots overlapped**

Image transparency
With this option (sometimes called dual channel display) turned on, each of the images is displayed in one of two colors, red and cyan (Figure 6-7). When the pixel colors of the two superimposed gels are added:

- Overlapping spots appear as shades of gray.
- Red spots are present only in the current gel.
- Cyan spots are present only in the specified reference gel.
- Halos of red or blue around dark spots indicate that the protein is over or under expressed, respectively, compared to the chosen reference gel.

This means that the less color you see in the Transparency mode, the more similar the gels. Of course, this is only true if the gels are correctly aligned (see Section 6.3.6) and superimposed.

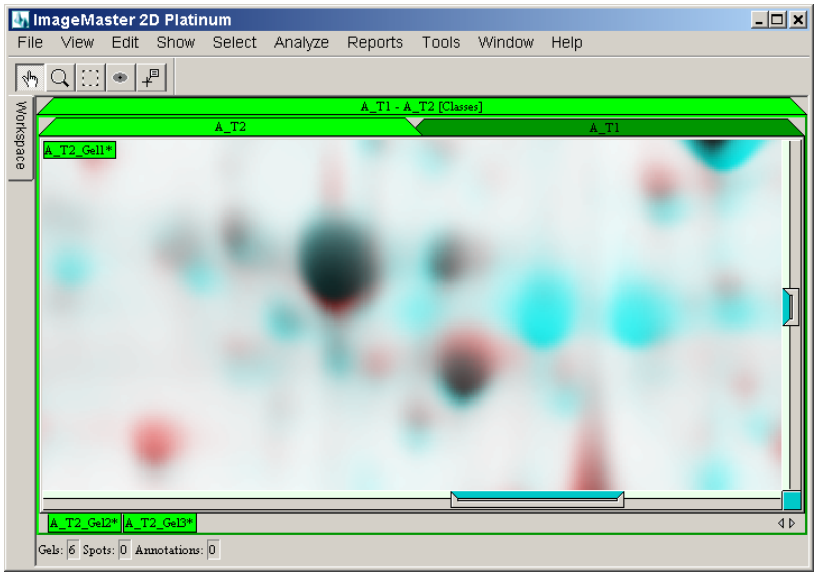


Figure 6-7. Image transparency.

Spots overlapped

With this option, when spots have been detected (see Chapter 7), the visible spots of the specified reference gel are displayed in blue on the image (Figure 6-8). Thus, you can easily compare the position and size of the red spots in the current gels, with the blue spots from the reference gel.

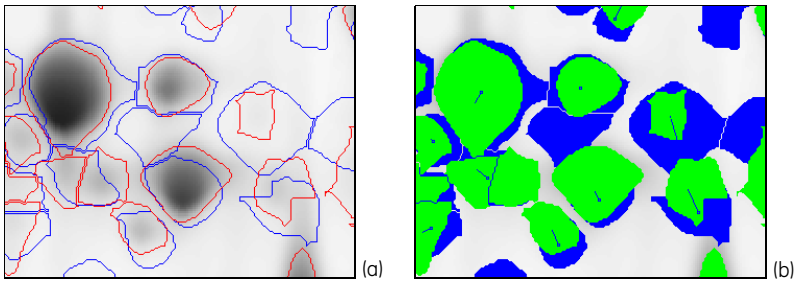


Figure 6-8. Spots overlapped with (a) outlined spot shapes and (b) filled spot shapes (selected spots in the front gel and pair vectors are displayed).

To activate the Transparency mode in the active worksheet:

1 Choose *Show* > *Gels* > *Transparency* > *Show* in the menu.

2 In the *Transparency Settings* window, specify the reference gel, check/uncheck the desired options and click *OK*.

3 ImageMaster displays your gels with the chosen options.

NOTE! You can first activate the Transparency mode and then align your gels with respect to the reference gel. You can even add landmarks in the Transparency mode. Nevertheless, it is recommended to perform any operations related to gel alignment (especially the definition of landmarks), before entering the Transparency mode to avoid a slow down due to the recalculation of the overlaid images.

You can change the default color to be used for displaying the spots from the specified reference gel (Overlapped spots). You can do this by going to the *Display* tab in *Tools* > *Options*.

6.3.5 Grid lines

Display *Grid Lines* over selected gels to indicate their dimensions in pixels (**Image coordinates**). Alternatively, you can show grid lines with **pI/MW** units, provided such information is available in the annotation category pI_MW. The pI/MW grid can also be displayed over gels that do not contain this information, but that were matched against a master gel having pI and MW values. The grid values are then calculated relative to the master gel.

The software will try to partition the visible area in the **Number of subdivisions** entered by the user. The graduations can just be regular subdivisions of the visible space - in **Fixed** mode - or a subdivision in terms of real coordinates at whole multiples - in **Adaptive** mode. Figure 6-9 shows various grid types, drawn as gray lines on the gels.

To display grid lines:

1 Select the gels.

2 Choose *Show* > *Gels* > *Grid Lines* > *Show* in the menu.

3 In the *Grid Settings* box, specify the **Type** of grid lines to be drawn (**Adaptive** or **Fixed**).

4 Specify the desired grid **Units** (**pI/MW** or **Image coordinates**).

5 Enter the number of horizontal and vertical subdivisions using the scrollbars.

6 The grid lines display over your gels (Figure 6-9).

7 To hide the grid lines again, select the gels and choose *Show* > *Gels* > *Grid Lines* > *Hide*.

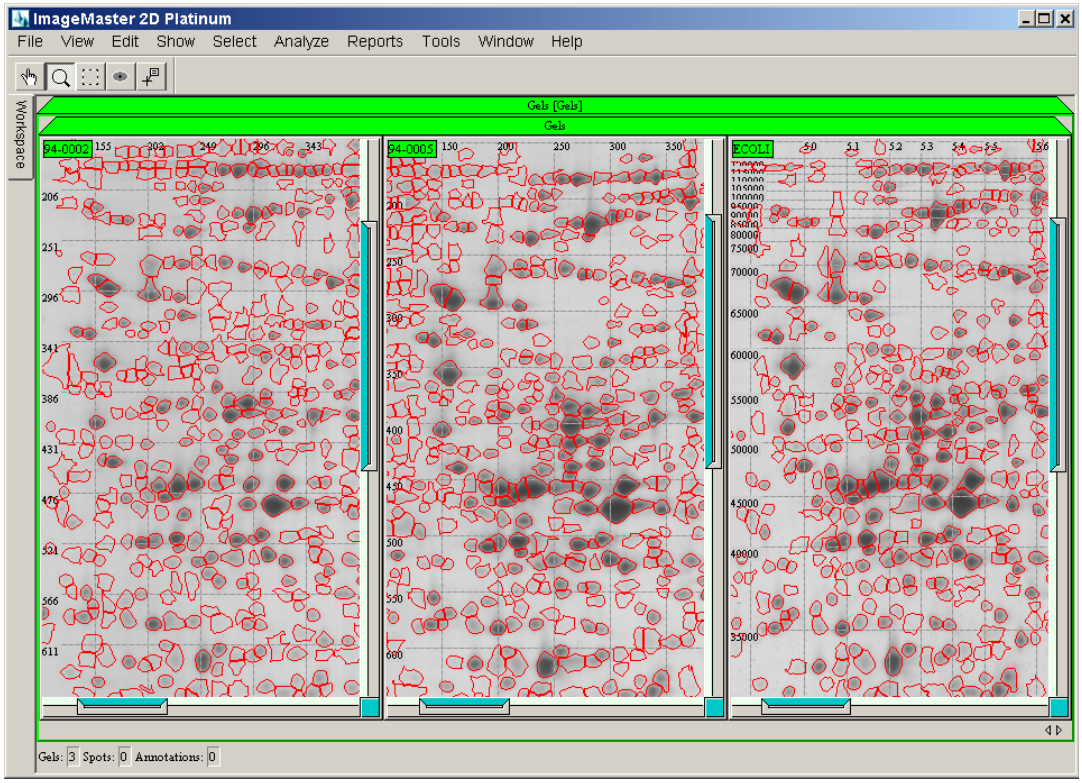


Figure 6-9. Grid lines displayed over gels. The left gel shows grid lines in Image coordinates/Fixed mode, the middle gel uses Image coordinates/Adaptive mode and the right gel displays a grid in pI/MW units/Adaptive mode.

NOTE! Displaying grid lines is a helpful way to visualize deformations in aligned gels (see below for details about aligning images) because the grid lines are warped in the same way as the image.

6.3.6 Aligning gels

The *Align* feature facilitates the visual comparison of images (stacked images in particular) that demonstrate large variations in protein migration. In fact, gel alignment essentially warps a gel so that it superimposes better with another one, thus allowing easy identification of corresponding spots (Figure 6-10). When aligning gels in a [MatchSet] or [Classes] worksheet, the selected gels are automatically aligned to the Master (see Chapter 9 to learn more about master images). When aligning gels in a [Gels] worksheet, a reference gel must be specified.

Gel alignment is purely a visual tool. It is not used in, and will not improve, the matching process. Therefore, the only reason to align your gels is to ease their visual comparison (possibly in combination with the Transparency mode).

To align a selected gel to a master image or specified reference gel, ImageMaster needs to know which positions in the different gels correspond to each other (represent the same protein form). This is done by defining landmark annotations on both the gel to be aligned and the master/reference gel. The alignment algorithm then deforms the aligned gel to superimpose those annotations that bear identical labels (in the master/reference gel and the aligned gel). Please see Chapter 8 to find out how to create such annotations.

NOTE! To carry out an alignment, annotations do not need to be linked with spots, and therefore spots do not need to be detected. This is what distinguishes alignment from matching. In matching, the landmark annotations must be linked to detected spots.

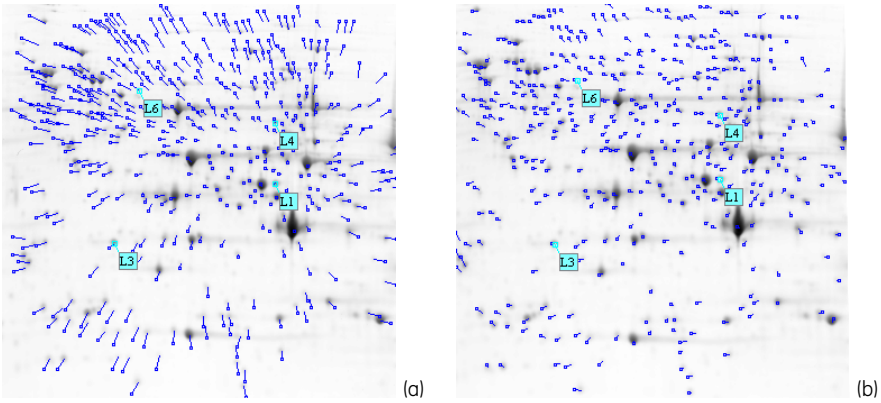


Figure 6-10. Gel (a) before and (b) after alignment with the master image. The match vectors and Landmark annotations are displayed.

ImageMaster allows you to choose one of the following alignment functions:

- **Global**: Authorizes the rotation, translation and scaling of the entire gel image to find the best possible correspondence between all the landmarks.
- **Exact**: Locally warps the image to exactly superimpose all the landmarks.
- **None**: Unaligns and restores the images.

Note that the addition of suspect landmarks or a bad distribution of the landmarks can worsen the alignment results. Therefore, these mistakes should be kept to a minimum, and the following rules considered when defining landmarks:

- For alignment using the *Global* option, landmarks should be well distributed over the entire gel. This means that they have to cover both the X and Y directions. On the other hand, when using the *Exact* option, landmarks should especially be defined around the distorted regions of the gels.
- Landmarks should only be defined on clearly corresponding spots. Moreover, one should differentiate between spots that effectively represent the same protein form, and spots that could be linked based on biological arguments (variants of the same protein such as different phosphorylation states). Protein variants definitely should not be used as landmarks.
- Landmarks should be placed on small, sharp spots (of similar area), rather than on large diffuse ones (which may differ considerably in size) because in the latter case the error in the position will be much more substantial.
- Landmarks should be added gradually, so that you can monitor their individual influence on the alignment, which is automatically updated after each landmark addition. Incorrectly placed landmarks may seriously decrease the alignment quality. If you add all your landmarks at once, you will not be able to discern which one poses a problem.
- When a spot is missing on one gel (sometimes happens to border spots), you should not put a landmark in a hypothetical spot position. Missing landmarks are not a problem for ImageMaster, as the program just aligns the landmarks that have the same name and ignores the rest.

To align two or more gels:

1 Bearing in mind the rules enumerated above, define a few annotations containing labels from the Landmark category on the gels. The master/reference gel for alignment and other gels should contain the same landmarks, that is, an identical label inside the same spot.

2 Select the gels to be aligned (include the reference gel when working in a [Gels] worksheet).

3 Choose one of the options in the *Show* > *Gels* > *Align Images* menu (*Global*, *Exact*).

4 Specify the reference gel if asked for (only in a [Gels] worksheet).

5 The selected gels are aligned relative to the master/reference gel.

6 If some parts of your gel are still not sufficiently aligned, you can add extra landmarks to your gels. The alignment is automatically updated.

7 To restore to the original images, choose *Show* > *Gels* > *Align* > *None*.

8 The original images are restored in place of the aligned ones.

In the event that gels are not aligned but have common landmarks, operations such as simultaneous gel moving, gel matching, etc. are done based on a simple interpolation between the two nearest common landmarks (landmarks with the same name). Otherwise, when the gels are not aligned and have no common landmarks, the operations are based on the same image location.

6.4 Viewing signal intensity

6.4.1 Loading / unloading gels

The digitized gel is composed of individual pixels, each of which is characterized by its horizontal and vertical positions (X and Y coordinates) and its signal intensity (raw pixel value).

The depth of an image (see Section 6.1.3) determines the number of possible pixel gray values. However, no matter what the original image depth, ImageMaster remaps an image to 256 gray levels for display. In the normal view of an image, raw pixel values do not need to be stored in memory. This corresponds to the default (unloaded) mode in ImageMaster.

For some operations such as spot detection, adjustment of the contrast mapping, and displaying the Profile, ImageMaster relies on the raw image data. The program automatically loads the pixel gray values before performing such an operation. When the operation is done, ImageMaster generally unloads the raw data in order to free up memory.

NOTE! The raw image data must be manually loaded in order to display pixel values in the *Cursor Information* window. The *Loaded* mode displays the original pixel gray values for the image. When your computer has limited memory, it is best to return to the *Unloaded* mode as soon as possible.

To load the raw image data:

1 Select the gels.

2 Choose *Edit* > *Gels* > *Raw Image* > *Load* from the menu.

3 No visible changes occur in the gel, but you may now display the raw pixel values in the *Cursor Information* window.

To unload the raw image data:

1 Select the gels. You can also choose *Edit* > *Gels* > *Raw Image* > *Select Gels with Loaded Image* in the menu.

2 Choose *Edit* > *Gels* > *Raw Image* > *Unload* in the menu.

3 The memory used to store image data is liberated and the raw pixel values are no longer available.

To load the raw image data every time a gel is opened:

1 Choose *Tools* > *Options* in the menu.

2 In the *Options* window, click on the *General* tab.

3 Check the **Keep image in memory** box.

4 The raw pixel values for all images are stored in memory by default.

The color depth of your computer screen (go to the *Start* > *Settings* > *Control Panel* > *Display* in the Windows menu for more details) affects the memory used by ImageMaster to display gels. For example, a display using 16 million colors (24 bits) requires three times more memory than one using 256 colors (8 bits). Therefore, you can also optimize memory usage in ImageMaster by adapting the color depth of your computer screen.

6.4.2 Cursor information

At any given time, the *Cursor Information* window can be used to display pixel values such as the X and Y coordinates or pI and MW estimates. When the original image data of the gels is loaded, the raw pixel values are shown. Note that if spots are detected, then the Cursor Information window also displays spot information.

To display cursor information:

1 Choose *Window* > *Cursor Information* in the menu.

2 In the *Cursor Information* window, click on the *Settings* icon to specify the information and display order (see Section 5.4.1 for more details on how to change, save and load settings). Click *OK*.

3 Position the cursor over the pixel you want. The *Cursor Information* window displays the related information. If the **Value** field is unloaded, the raw pixel values are not available. In order to see the raw image data, choose *Edit* > *Gels* > *Raw Image* > *Load* in the menu.

6.4.3 Displaying gray levels

A 2-DE gel image is traditionally displayed as a gray level image, where gray levels represent the signal intensity. Sometimes the displayed gray levels are so low that small spots are hardly visible. In order to emphasize these very faint spots, you can adapt the brightness and contrast of the image. You can also display images using pseudo colors. These types of operations are carried out with the *Adjust Contrast* function.

Generally, it is useful to preview the modifications that will be done to your image. To get such a preview, it is sufficient to select an interesting region in your gel. This can be done before and during the use of the Adjust Contrast feature. In fact the *Adjust Contrast* window can be left open throughout your work session, so that you can adapt parameters at any time in order to highlight faint spots or to help in spot editing decisions. This means that you can continue to select gels and other objects while the *Adjust Contrast* window is open, and that you can move the region in your gel at any moment to locally increase the contrast for better viewing. As you will see below, the Adjust Contrast function may be more interesting in the preview mode than when its modifications are simply applied to the gels.

NOTE! Any changes done by the Adjust Contrast function only influence how the image is displayed on your screen, and do not affect the underlying data, spot detection and quantitation.

6.4.4 Contrast mapping

As mentioned earlier, modern scanners are usually able to scan 2-DE images with 12 or even 16 bits per pixel, 4096 or 65536 gray levels, respectively. Because common computer screens are only able to display 256 gray levels, mapping must be undertaken between the 4096 (or 65536) image gray levels and the 256 screen gray levels. By default, ImageMaster uses a linear mapping function,

where the lightest point in the image is mapped to 0 (white) and the darkest point is mapped to 255 (black).

This is illustrated with the gray level histogram in Figure 6-11. The histogram displays the frequency with which each gray level (from 3524 to 28530) occurs in the gel image A_T1_Gel1. The low gray levels (at the left of the histogram) corresponding to the background occur very often, whereas the high gray levels (at the right of the histogram) corresponding to the darkest spots are much less frequent. The red line indicates that the minimum and maximum gray levels (3524 and 28530, respectively) are remapped by the default linear mapping. The vertical axis for the red remapping graph corresponds to the 256 screen gray levels.

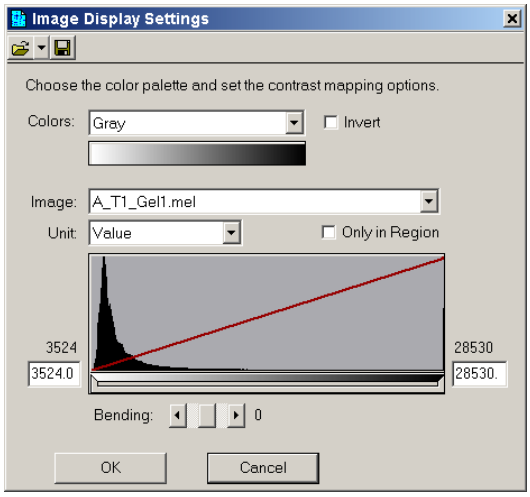


Figure 6-11. Adjust contrast function.

However, you can choose other mapping functions to accentuate small faint spots. ImageMaster offers two ways to change the default gray level mapping and thus improve the visual display of the gels.

You can define the minimum and maximum gray levels (i.e. look at only the light or the dark regions in the images). Do this by decreasing the size of the gray level range that is to be remapped linearly to the screen gray levels. To accomplish this, move the left or right borders of the slider that is found below the histogram function. Once the size (interval) of the slider is decreased, you can also displace the interval by placing your cursor in the middle of the slider, holding down the left mouse button, and moving it to the left or right.

In Figure 6-12, the histogram shows the gray level distribution of the selected image. The maximum gray level was set to 9398 by moving the right side of the

slider to the left. The red transformation function shows that gray level 9398 is now mapped to the maximum screen gray level (255 or black), and that any darker pixels will appear as black.

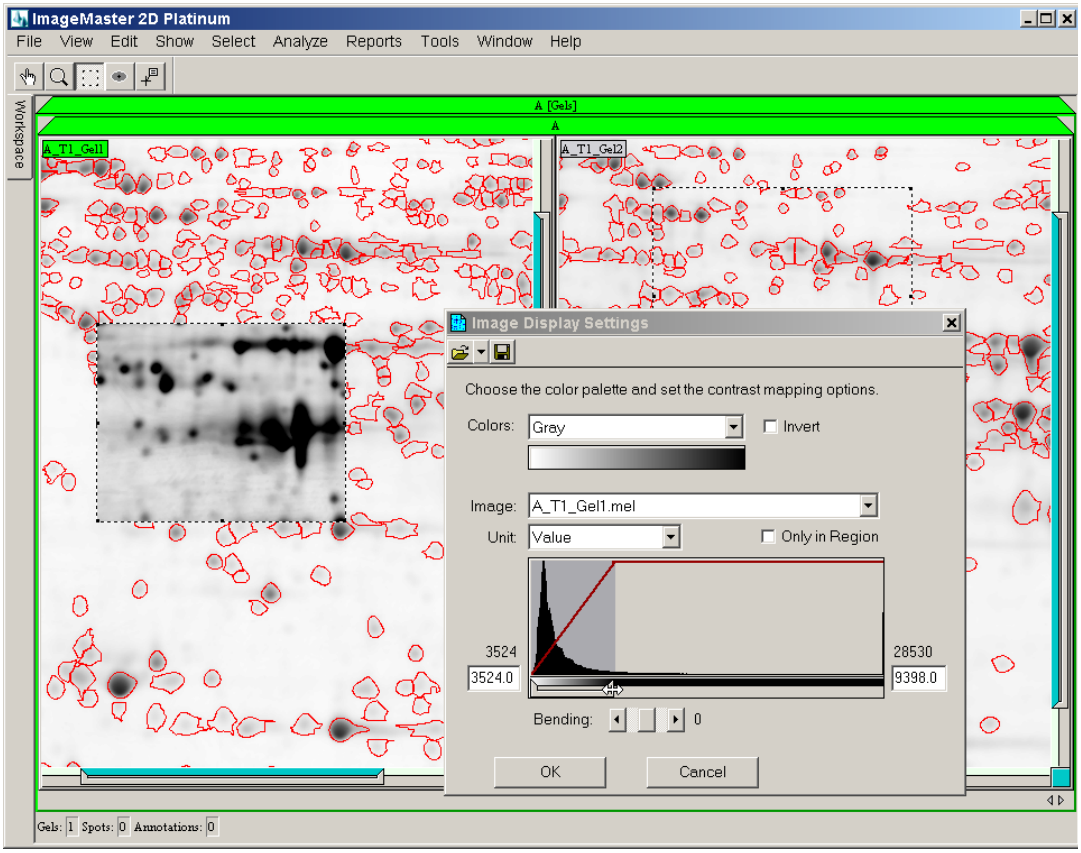


Figure 6-12. Remapping of the gray levels. Adjustments are immediately reflected in the preview region.

Another way to change the default gray level mapping is the use of a non-linear mapping function. The **Bending** parameter expands or compresses the contrast range at the dark or light ends of the range. When the bending parameter is positive, the image is lighter. The image is darker when the bending parameter is negative (Figure 6-13).

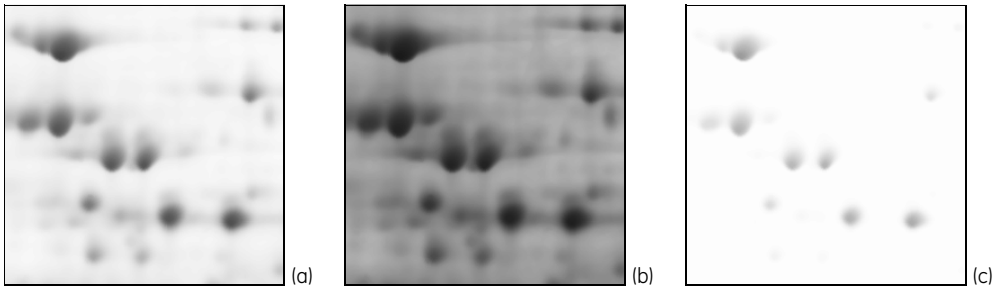


Figure 6-13. Region of a gel showing a contrast adjustment. (a) Original image with bending parameter 0, (b) same area with bending parameter set to -2, (c) same area with bending parameter set to 2.

To adjust the contrast in an image:

1 Select one or more gels. Draw a region in these gels to get a preview of the contrast mapping modifications.

2 Choose *Show* > *Gels* > *Adjust Contrast* in the menu.

3 In the *Image Display Settings* box, select an image from the list. The grey level histogram of the chosen image is shown and can now be used for adjusting the contrast.

4 Specify the **Unit** you want to use (see below) and indicate whether you want to modify the contrast mapping as a function of the complete image range or only of the selected region (see below).

5 Change the minimum and maximum gray levels by displacing the slider borders or by typing valid numbers in the boxes at the lower left and right corners of the histogram. You can also use the bending scroll bar.

6 These settings are applied to the regions in the selected gels.

7 When you are satisfied with the changes, click *OK* to apply the settings to all the selected gels.

All contrast mapping changes are applied to selected gels only and are saved with the image file. Thus, the next time you open these gels, the modifications are still active.

In some cases, you may want to save a specific mapping function so that it can be applied at a later time to other gels with similar gray level properties. To do this, click on the *Save* icon in the *Image Display Settings* window and enter a file name as well as the desired destination folder. ImageMaster saves the mapping

function with the extension .glt (Gray Levels Transformation). The mapping functions stored in the default folder (*ImageMaster\Menus* folder in the user's *My Documents* directory) are always available from the *Open* icon in the *Image Display Settings* toolbar. It is possible to load the gray mapping function of any open gel by selecting the gel's name from the *Current* list in the dropdown menu attached to the *Open* icon in the *Image Display Settings* window.

Unit
Two different units are used for displaying the gray level minimum and maximum:

- **Value**: Uses the raw pixel values as displayed in all the reports. When a calibration is done, these correspond to the calibrated pixel values.
- **Percentage (%)**: Chooses the scale as a percentage of the total gray level range in the histogram.

Only in region
By checking the **Only in Region** box in the *Image Display Settings* window, ImageMaster only considers the gray levels that are present in the selected region.

Only in Region is very useful in combination with the **% Unit** and the choice of a relatively small region. In this case, you enter an adaptive mode that allows you to adjust to the local gray levels. The effect is a local increase in contrast that is very useful for viewing very faint spots. This mode can also be combined with the **Gray+Saturation** palette in the **Colors** list.

Pseudo colors
You may want to use a particular color palette instead of the standard gray levels in your gel. ImageMaster offers color palettes such as **Coomassie Blue**, **Silver Stain**, **Hot Iron**, **Spectrum**, etc. A palette that is especially useful is the **Gray+Saturation** palette. It corresponds to the standard **Gray** option, except for the maximum value that is displayed in red, and the minimum value that appears in blue. It is helpful when you want to visualize saturated spots (red) or the background (blue). Of course, you can modify the minimum and maximum gray levels, as explained above, to decrease the stringency on what is considered as saturation or background. Finally, you can just inverse the gray levels by checking the **Invert** box.

Please note that any color adjustments and the inversion of the gray levels using the Adjust Contrast function are applied to all selected gels and are not saved with the gel images.

To display gels with pseudo colors:

1 Select one or more gels. Draw a region to get a preview of the color adjustment or inverted gray level modifications.

2 Choose *Show* > *Gels* > *Adjust Contrast* in the menu.

3 In the *Image Display Settings* box, select one of the color palettes in the **Colors** list and/or invert the gray levels by checking the **Invert** box.

4 The modifications are applied to the defined regions in the selected gels.

5 If you are satisfied, click *OK* to apply the changes to all gels.

6 To go back to the default gray levels, choose the **Gray** option from the **Colors** list.

6.4.5 Profile

It is sometimes quite difficult to judge whether spots should be split, whether they are saturated or not, or whether they have some other problems. Sometimes spots may have a so-called donut structure, with low intensities in the center compared to the borders. It is very important to identify such problems, as they will lead to incorrect spot quantitation. However, such errors are difficult to detect because the human eye is not very sensitive to different shades of gray.

The *Profile* function can help in such cases. Horizontal and vertical sections on the gel at the position of the mouse cursor show the intensity variations in the two directions (Figure 6-14). The Profile visually clarifies the intensity changes in the gel and can thus assist in making decisions.

Please note that the major advantage of the Profile feature, compared with the 3D View (see below), is that it can be used within the selected image during spot editing and so on. Thus, you have information at your disposal about the third dimension (spot intensity) without having to open an additional window, as is the case with the 3D View option.

ImageMaster relies on the intensity of every pixel in the gel to display the Profile. For this reason, the raw image data are automatically loaded when using this utility. If the memory resources on your computer are low, please do not forget to unload the raw image data when you finish using the Profile function (see Section 6.4.1).

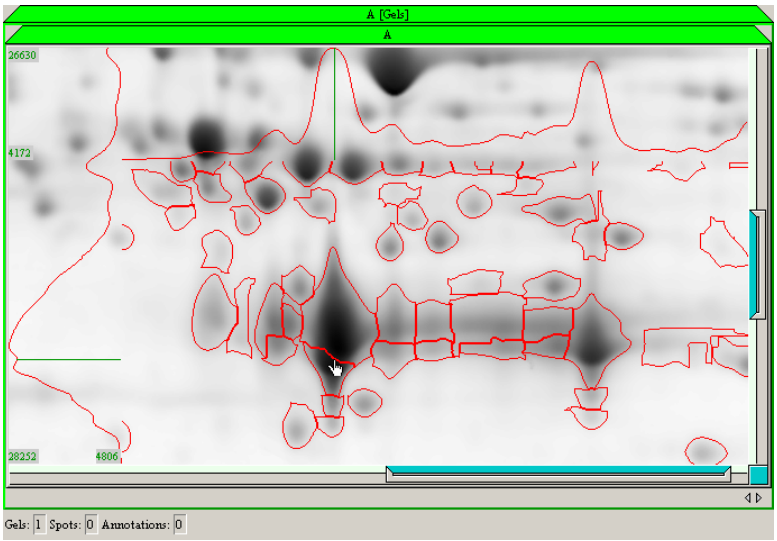


Figure 6-14. Profile feature. Red curves represent the intensity variations of the gel in the vertical (left) and horizontal (top) directions, at the position of the mouse cursor. Green lines indicate the exact position of the cursor, whereas the numbers indicate the minimum and maximum gray levels in a specific Profile view.

To view profiles on your gels:

1 Choose *Show* > *Gels* > *Profile* in the menu.

2 Position your mouse cursor over a gel. The horizontal and vertical profiles at the cursor position are displayed.

3 Once the *Profile* feature is no longer needed, choose *Show* > *Gels* > *Profile* from the menu.

6.4.6 3D view

Another way to examine the intensity variations in a gel is by looking at the three-dimensional (3D) view of a gel region (Figure 6-15). In this type of view, the X and Y axes represent the pI and MW values, whereas the pixel intensity is plotted along the third dimension (Z axis). The resulting image shows a peak for each protein spot, with a peak height that is proportional to the spot intensity. It can be rotated in any direction to view the interesting spot(s) from all sides, thus facilitating spot editing or matching decisions.

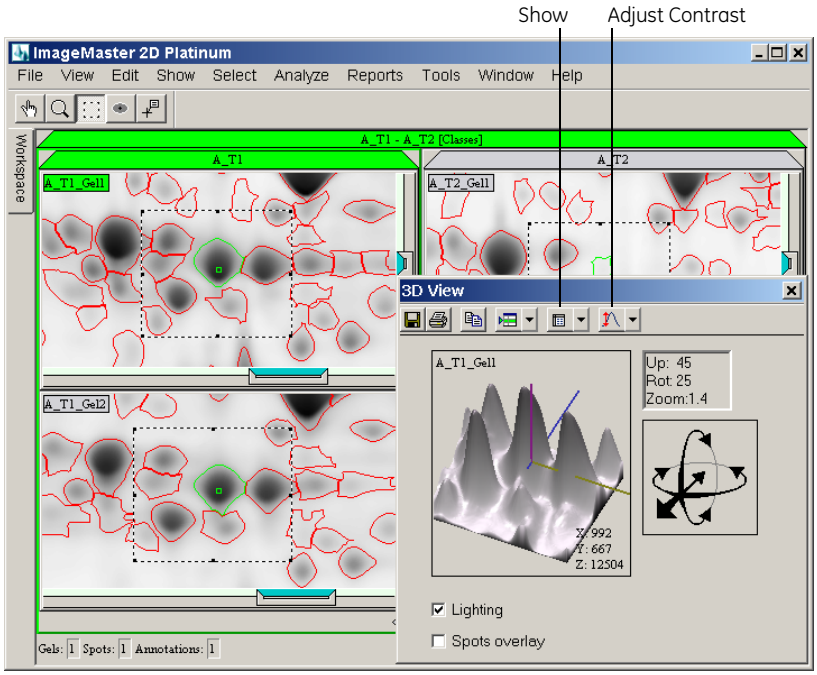


Figure 6-15. 3D View for the active region of gel A_T1_Gel1.

To display the 3D View of a gel region:

1 Select a region in one or more gels. Or select one or more spots.

2 Choose *Reports* > *3D View* in the menu.

3 When both spots are selected and a region is defined, ImageMaster will ask whether your *3D View* should be based on the selected region or selected spots.

4 ImageMaster displays a *3D View* for the region or the area containing the selected spots.

Figure 6-15 shows the region in gel A_T1_Gel1 and the corresponding *3D View* window. At the top of this window, you see the typical toolbar. You can turn the gel by clicking on the arrows at the right side of the gel image. By default, the three axes are displayed: the X axis in brown, the Y axis in blue, and the Z axis in purple. You can change the point on which the axes are centered by right clicking on the desired position. This position then becomes the new center of the image. Note that this is a way to move the view up or down, depending on the position you click on. The coordinates of this center point are found at the lower right corner of the image. The angles of rotation in the vertical and horizontal directions

as well as the zoom factor are displayed at the right side of the *3D View* window. Please note that vertical rotation is limited between 0° and 90° (you cannot look 'under' the gel).

Below the 3D image, the **Lighting** check box enables you to switch off the light sources that ensure a proper three-dimensional aspect of the image. The result is that the gel will appear like a normal gel image when it is looked at with a vertical angle of 90° (flat position) and that the individual pixels are visible.

NOTE! The pixel gray level is overlaid on the 3D image; the top of the peaks is therefore always darker. This also means that you can combine the 3D View with the various color palettes available through the Adjust Contrast feature.

The second box at the bottom of the *3D View* window, called **Spots Overlay**, should be checked when you want to display the spot positions on the view. Spots are shown with outlines corresponding to the spot borders or as crosses indicating the spot centers. To choose one of these options, you should go the *Spot Shape* item under the *Show* drop down menu of the toolbar.

The 3D View module in ImageMaster also enables comparative viewing of corresponding regions or spots in different gels (Figure 6-16). When using this feature, the various regions are displayed in a single window using identical scales and orientations, allowing direct comparison of spot positions and heights.

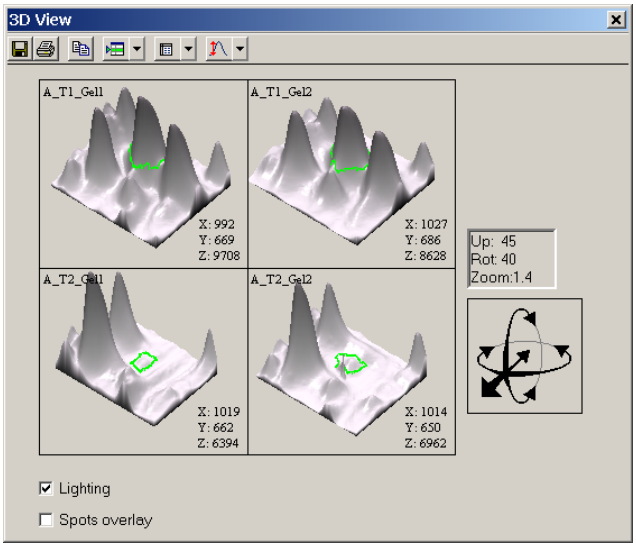


Figure 6-16. Multiple 3D views for the comparison of corresponding spots or regions in different gels.

To display multiple 3D views of several (corresponding) gel regions:

1 Use the Shift key to select the same region in all selected gels, or define a particular region for each gel. Alternatively, you can select one or more spots in each gel.

2 Select the gels for which you would like to display and compare the 3D views.

3 Choose *Reports > 3D View* from the menu.

4 When spots are selected and a region is defined, ImageMaster asks whether your *3D View* should be based on the selected region or highlighted spots.

5 ImageMaster displays a single window with side-by-side 3D views for each active region (or area around selected spots).

Zoom operations and rotations are applied to all gels in a multiple 3D View. Right clicking on the desired position can change the point on which the axes are centered. When holding the Shift key at the same time, this change is applied to all the 3D views in the window.

The 3D View is considered to be a normal report, and, as such, provides most of the standard icons in its toolbar. More information about the icon functionalities is found in Chapter 5.

In short, the 3D image can be saved in PNG, TIFF or BMP formats and printed or copied to the clipboard for insertion into another program. The *Select on Gel Image* icon makes it possible to highlight spots on the related gel image, once they have been selected in your 3D View. To select spots on the 3D View, just left click on their center or on the tip of their peak when viewed from the side. With the *Select on Gels+Reports* option in the *Select on Gels* drop down menu, the spots are not only selected in the corresponding gels but also in any open reports. Finally, the *Select from Gels* feature does the exact opposite, any spots selected on the corresponding gel region are highlighted in the 3D View.

The *Show* icon in the *3D View* toolbar groups a series of special features:

- **Show Region on Gel** causes the gel to be moved so that the region corresponding to the 3D View (and more particularly the center of the axes in the 3D View) becomes visible. This option is useful for finding a specific point on the gel when no spots were detected.

- **Set Region on Gel** not only moves the gel so that the region corresponding to the 3D View becomes visible, but also reselects the region on the gel. One

should be careful when using this option because any previously selected region is lost.

- **Synchronize all 3D Views** is used to propagate the orientation (for example, the **Up**, **Rot** and **Zoom** parameters) of the current view to all other 3D views. You can perform the same action by holding down the Shift key while adjusting the center point of the axes in one of the gels (by right clicking).
- **Spot Shape** sets the way the spots are displayed.
- **Display axes** can remove the axes from the 3D View.

The last icon, *Adjust Contrast*, enables you to quickly change the gray level setting of the peaks.

NOTE! An interactive version of 3D View is available from the *Window* > *Mouse Selection* menu. Its functionality is much the same as the standard 3D View, but it interactively displays the third dimension for the current mouse selection (region or spots). When you select a new spot on your gel, the 3D view (as well as that of matched spots in other selected gels) automatically displays. When you deselect the spot, its 3D view immediately disappears from the report.

6.5 Calibrating and normalizing gels

ImageMaster offers several possibilities to compensate for image differences caused by variations in experimental conditions (for example, protein loading or staining) and scanning properties (such as image depth). This section describes the available features. The terms normalization and calibration, as used within ImageMaster, are also clarified. Please note that you need to master the notions of spots and matches to fully understand the functionalities explained below. You can refer to the following chapters to learn more about these ImageMaster objects.

6.5.1 Intensity calibration

Instead of displaying and using the gray levels taken directly from the image capture device, you can assign real world values, often the optical density (OD), to the measured pixel values. The advantage of this is that the range of gray values is the same no matter what the original pixel depth. Some image capture devices automatically perform this type of calibration. When they do, ImageMaster generally reads the calibrated intensity values from the saved files. For example, images acquired with the GE Healthcare ImageScanner using the LabScan 5.0 software, are already calibrated.

Displaying calibration information

You have different ways of finding out if your images were calibrated and for viewing the calibration information:

- Choose *Reports* > *Gel Report* to view the calibration function (**Calibration**) and calibration units (**Unit**) for the selected gels. If nothing appears in these columns, the gels are not calibrated.
- Choose *Reports* > *Gel Calibration* > *Report* to display a report including the calibration function, the name of the step tablet used, the name of the person that did the calibration, and the calibration date.
- If your gel has been calibrated with LabScan 5.0, you can select the gel and choose *Reports* > *Gel Calibration* > *Plot* to view the calibration curve. See below for details about the calibration curve.

Creating a calibration

If you intend to calibrate the image capture device yourself, you need to scan a calibration step tablet or calibration strip along with your gels. These step tablets have known intensity values (expressed in optical density, OD, or diffuse density, DD) published by the manufacturer of the step tablet. Please note that for the purpose of 2D gel analysis, it is only useful to calibrate the image capture device when working in transparent mode. Normally no calibration needs to be done when you do reflective scanning. With some equipment, both a transparent and a reflective calibration strip are provided. When calibrating, be sure to use the appropriate calibration step tablet.

NOTE! The OD values for the step tablet have to be specified in a **Calibration Tablet File**, together with other information such as the height and width of the tablet, and the number of steps. An example of such a Calibration Tablet File (Kodak2.tab) can be found in the *Template**Tablet* folder of the ImageMaster installation directory. This Calibration Tablet File is made for use with the Kodak Step Tablet no. 2. If you do not use this specific step tablet, you can copy the file and edit the data to make your own Calibration Tablet File. You can edit the file with tools such as Windows Notepad.

As the intensity values supplied with your step tablet are generally expressed in diffused density (DD), you have to convert them to OD values. For this purpose, the manufacturer of the step tablet should provide the appropriate relationship. For the Kodak Step Tablets no. 2 and 3, for example, this is OD = 1.4 DD.

To create a calibration once a correct Calibration Tablet File is generated:

1 Scan the step tablet and import the image file into the ImageMaster software. If necessary, rotate the image such that the light steps are displayed at the top.

2 Choose *Tools* > *Calibration Tablet* > *Create* in the menu.

3 The *Load Step Tablet Definition* box opens. Browse the folder where you saved the **Calibration Tablet File** specifically tailored to your step tablet (see above) and *Open* the file.

4 A red calibration step overlay appears on the image and the *Create Calibration* window is displayed (Figure 6-17).

5 Select the *Spot* or *Annotation* tool in the ImageMaster toolbar and adjust the position of the steps by dragging the overlay while holding the left mouse button. To help you differentiate the darkest steps, you can adjust the contrast. Please note that the size of a step on the red overlay (not the red box, but the distance between two short horizontal lines) should correspond exactly to the size of a step on the image. If this is not the case, you must adjust the height of the tablet in the **Calibration Tablet File**.

6 At the left of the *Create Calibration* window, you see the theoretical optical density (OD) values of the different steps in the tablet (the values you entered in the **Calibration Tablet File**). ImageMaster may automatically deselect some of the steps (grayed out) because of their unreliable values, and you can deselect additional ones if you estimate that they should be excluded from the calibration process. At the right of the *Create Calibration* window you see the calibration curve between the logarithmic transmittance values on the X-axis, and the OD intensities on the Y-axis. Note that the measured intensity values for each step are calculated as median intensities over all the pixels in the small rectangle area for each step (on the step overlay). The horizontal dispersion intervals in blue (or gray for deselected spots) represent the intensity ranges when 10% of the less intense and 10% of the most intense pixel values are removed. The calibration formula and error are given below the graph.

7 You can additionally display some reports to judge the quality of your scanner calibration (see below for more details).

8 Once you are satisfied with the calibration, close the *Create Calibration* window. The software asks whether you want to apply this new calibration. If you answer *Yes*, ImageMaster applies the calibration to the image.

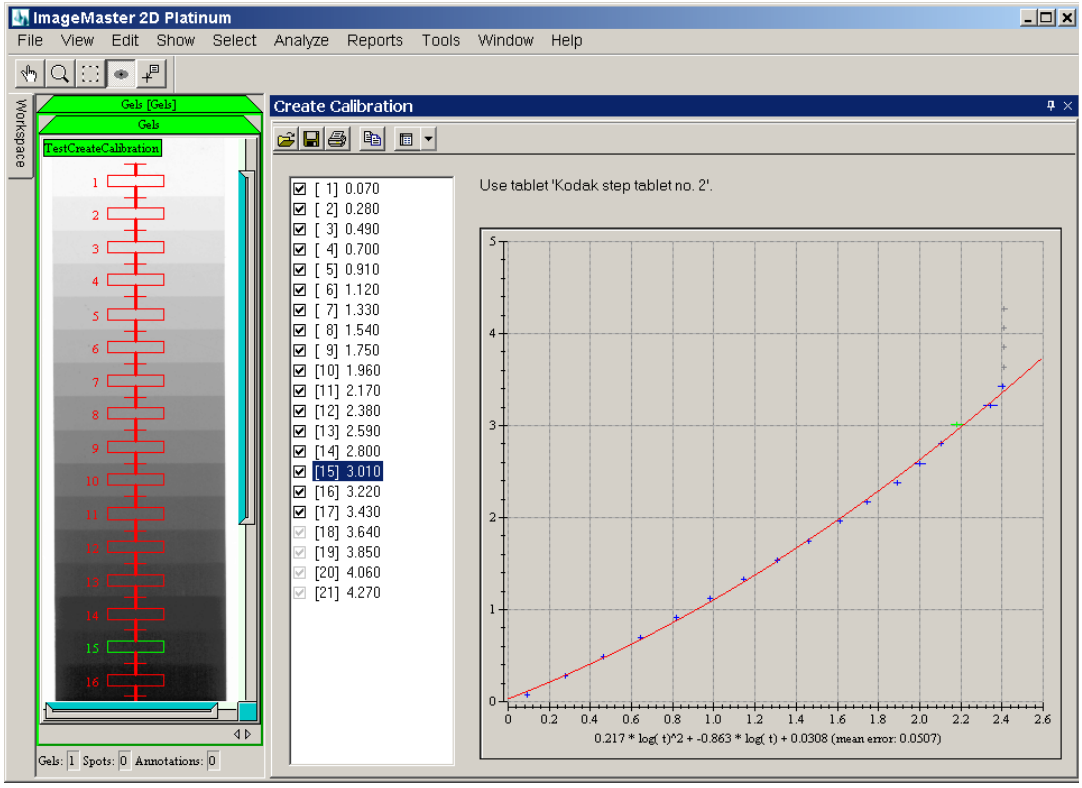


Figure 6-17. Image of the step tablet with the red calibration step overlay. The Create Calibration window shows the calibration curve and the OD values for the different steps (on the left).

NOTE! When you select a point in the step tablet list (in the *Create Calibration* window), the corresponding step becomes automatically highlighted in green on the step tablet overlay and in the calibration graph. This makes it very easy to correlate the three sources of information (point in tablet list, point in graph and step rectangle).

The various icons in the toolbar of the *Create Calibration* window are used to:

	Open another tablet definition.
	Save the calibration (with the extension .cal).

	Print the calibration graph.
	Copy the calibration graph to the clipboard.
	Display related **Reports**.

Two reports are available from the *Reports* icon in the *Create Calibration* window. These reports can be saved, printed and copied to the clipboard.

- The **Fitting Report** displays the coefficients of the regression function.
- The **Calibration Report** displays for each step: the step number, the measured averaged gray level, the calibrated intensity value, and the fitting error (difference between the curve and the point).

NOTE! Intensity calibration must be performed on a regular basis (once a month). If you do not calibrate, darker material may not be measured in the correct proportions to the lighter material.

Controlling a calibration

The *Control* calibration mode allows you to verify whether you are using a correct calibration. It requires a different, specially calibrated step tablet (Kodak Step Tablet no. 3, for instance), which you compare to your previous calibration results. So you have a calibration step tablet for everyday use, and a specially calibrated control step tablet to verify your calibration periodically.

To control a calibration:

1. Scan your control step tablet (Kodak Step Tablet no. 3, for instance) and import the image file into the ImageMaster software. If necessary, rotate the image such that the light steps are displayed at the top.
2. Choose *Tools* > *Calibration Tablet* > *Control* in the menu.
3. You are asked to load the calibration to be controlled. This calibration could have previously been saved using the *Save* icon in the *Create Calibration* window (.cal), or can simply come from a calibrated image file (.mel) such as the calibrated step tablet image obtained in the section above.
4. Next load the definition of the control step tablet. This **Control Tablet File** must be specifically adapted to this new step tablet. That is, it should have been edited with a tool as Windows Notepad so that it contains the

appropriate OD values, height, width and number of steps corresponding to the control step tablet.

5 The red calibration step overlay appears on the image of the control step tablet and the *Control Calibration* window is displayed.

6 Select the *Spot* or *Annotation* tool in the ImageMaster toolbar and adjust the position of the steps.

7 You should now verify that the calibration curve is passing through the data points correctly and with minimum dispersion intervals. If this is not the case, try to find out why your current calibration does not seem to work properly.

Applying a calibration

To apply a calibration to newly scanned gel images:

1 Open and select the gels the calibration should be applied to.

2 Choose *Edit* > *Gels* > *Apply Calibration* in the menu. Select the source of the calibration information. This can be an open gel (or step tablet image) that was already calibrated, or you can select a file (.cal or .mel) from the hard disk.

All pixel and spot values subsequently displayed in any reports or in the *Cursor Information* window correspond to the calibrated values.

You can remove a calibration from a gel. Note that this can also be done for gels that were already calibrated when you imported them into ImageMaster.

To remove a calibration from a gel:

1 Select the gels from which you want to remove the calibration.

2 Choose *Edit* > *Gels* > *Reset Calibration* in the menu.

3 You are asked to confirm your choice.

6.5.2 Spot normalization

Spot normalization is a kind of internal calibration that makes the data independent of experimental variations between gels caused by conditions such as differences in protein loading or staining. In ImageMaster, this simply implies the use of the relative Intensity (%Intensity) or relative Volume (%Vol) to quantify and compare the gel spots. By definition:

$$\%Intensity = \frac{Intensity}{\sum_{S=1}^{n} Intensity_S} \times 100$$

where $Intensity_S$ is the calibrated intensity of spot S in a gel containing n spots.

$$\%Vol = \frac{Vol}{\sum_{S=1}^{n} Vol_S} \times 100$$

where Vol_S is the volume of spot S in a gel containing n spots.

These measures take into account variations due to protein loading and staining, by considering the total intensity or volume over all the spots in the gel. This means that in a gel where, globally, the spots are darker than in another image, the majority of spot volumes is higher. However, the bulk of %Vol should be similar to those in the compared image, at least for gels with similar spot patterns.

Please note that although the %Vol is a rather efficient measure for evaluating protein expression differences between gels, the %Intensity is not as relevant to use.

6.5.3 Intensity normalization using a scatter plot

Intensity normalization may in some cases be beneficial or even indispensable to obtaining more precise differential expression values. However, if your experimental procedure is highly reproducible, this image compensation method is not always needed. Many differential protein expression studies yield good results without requiring intensity normalization. Improper usage of this tool can even be detrimental to your analysis. Therefore, clearly understand the assumptions that are made in the application of this feature before you decide to use it on your specific set of gels.

The scatter plot in ImageMaster renders information about the relationship between the spot values from two gels by searching for the linear dependence between the values from one gel and the values from another gel (see Section 10.2.1). In numerous cases only a relatively low percentage of the spots in the compared gels are expressed differently. Most of the spot values (especially Intensity values) evaluated in the two gels should be similar, and therefore the best-fit line of the scatter plot should be close to identity (Y=X). This assumption permits your gels to be normalized based on the best-fit line. That is, to correct the pixel values in one of your gels in such a way that the best-fit line approaches identity. This normalization therefore minimizes the number of spots that are expressed differently, and can be used to compensate for sample loading variations.

To normalize intensities based on a scatter plot:

1 Select the gels that might need normalization, including the reference gel to be used for the scatter plots. The gels and the reference gel should be matched (belong to the same match set).

2 Select all matches.

3 Display the scatter plots by choosing *Analyze* > *Intra-Class* > *Scatter Plots*. Make sure you choose **Intensity** as value type. This is important because normalization does not work with other value types.

4 If the slope in a scatter plot is close to 1, and the offset close to 0, then normalizing the data is not appropriate. However, if these values are not close to 1 and 0, then try normalizing your gel using the following procedure.

5 Select the scatter plots of the gels that need to be normalized.

6 Choose the *Fitting Report* option from the *Reports* drop down menu in the toolbar of the *Scatter Plots* window, and save this Fitting Report. The Fitting Report gives the gray slope, gray offset, and correlation values for the scatter plot best-fit line for each gel as well as the number of matches that were selected to display the scatter plot. If you only select some of the lines in the report, you will get the chance to save just those in the file.

7 Select only the gels to be normalized (make sure the reference gel is deselected).

8 Import the normalization by choosing *File* > *Import* > *Normalization* from the menu and selecting the previously saved fitting report. Two different scenarios can occur. If the fitting data of a single gel was saved in the report, the normalization will be applied to all or none of the selected gels, depending on your choice. If the fitting data for two or more gels was saved, each normalization in the file is applied to the gel with the corresponding name.

9 Alternatively, you can select the gels one by one. Choose *Edit* > *Gels* > *Edit Normalization* in the menu, and enter the appropriate **Gray Slope** and **Gray Offset** values (corresponding to those from the scatter plot for the particular gel).

10 Each selected gel is now normalized. To check this, you can display the new scatter plots. All slopes should be close to 1 and all offsets close to 0. Normalized gels will have an asterisk after their name in various reports and displayed spot values correspond to the normalized values.

11 You can remove the normalization at any time by choosing *Edit* > *Gels* > *Reset Normalization* in the menu.

Please use caution with respect to this method because if there are many proteins that are expressed differently, then you can introduce errors and get identical values for corresponding spots that are in fact widely divergent. This type of normalization should essentially be restricted to gels that belong to the same population or that represent the same sample.

For gels with very different protein patterns that present significant disparities in protein loading and/or staining, you may be able to carry out normalization on the condition that both gels contain a series of internal standards (proteins for which the abundance is known to be identical in the two gels). In this case, you can perform scatter plot normalization as long as only the spots corresponding to the internal standards were selected when displaying the plot.

6.6 Processing gels

A certain number of functionalities in ImageMaster not only imply the processing of existing 2-DE images on screen, but also affect the image files on the hard disk. These options are grouped in the *Tools* > *Gels* menu and lead to the creation of new gels (except for the *Delete from Disk* and *Rename* option) that are automatically opened and displayed in the ImageMaster window. The newly created gels are also inserted in the Gels folder of the current project in the Workspace. Some features, such as cropping gels, are not provided in the *Tools* > *Gels* menu because they must be carried out in other ways. Nevertheless, the processing operations are described below.

6.6.1 Delete from disk

With this function, you not only remove your gels from the screen but also from your hard disk. The main advantage of deleting gels from within ImageMaster is that associated matches are suppressed as well.

To delete existing gels:

1 Select the gels to be deleted in a worksheet.

2 Choose *Tools* > *Gels* > *Delete from Disk* in the menu.

3 All selected gels and their associated match files are closed and removed from your hard disk.

6.6.2 Renaming gels

Since the ImageMaster Workspace checks the consistency between the gel Name and Gel ID, it is highly recommended to rename your gels (at least those

that have already been opened and saved with ImageMaster) from within the program. By doing so, you avoid inconsistencies in the workspace.

To rename your gels:

1 Select the gels to be renamed in a worksheet.

2 Choose *Tools* > *Gels* > *Rename* from the menu.

3 Enter a new gel name for each selected gel.

4 The gels are automatically renamed (gel legend, workspace and file name).

6.6.3 Rotating gels

When images are scanned in the wrong orientation, they can be rotated to correct for this. You can typically rotate your gels by 90, 180 and -90 degrees. But free rotation can also be applied.

To rotate an image:

1 Select the gel to be rotated in a worksheet.

2 Choose *Tools* > *Gels* > *Rotate* from the menu and select the desired option (*90° CW*, *180°*, *90° CCW*, *Free*).

3 Enter a new file name and destination folder for the rotated gel.

4 If you choose one of the predefined angles, the rotated image is directly saved to the disk drive and opened in the ImageMaster window.

5 If you select the *Free* rotation option, the image appears with a grid on it. The bold horizontal grid line plays the role of landmark to help you visualize the rotation. It becomes the new horizontal in your rotated image.

6 Make sure the *Region*, *Spot* or *Annotation* tool is activated. Then click on any position on the gel and rotate the grid while holding the left mouse button. Release the button when the bold line is parallel with what should be the new horizontal in your image (Figure 6-18). You can also manually enter a rotation angle in the *Rotation Tool* dialog box.

7 Press *OK* to confirm your rotation (spots and annotations are preserved) or *Cancel* to return to the initial orientation of the gel.

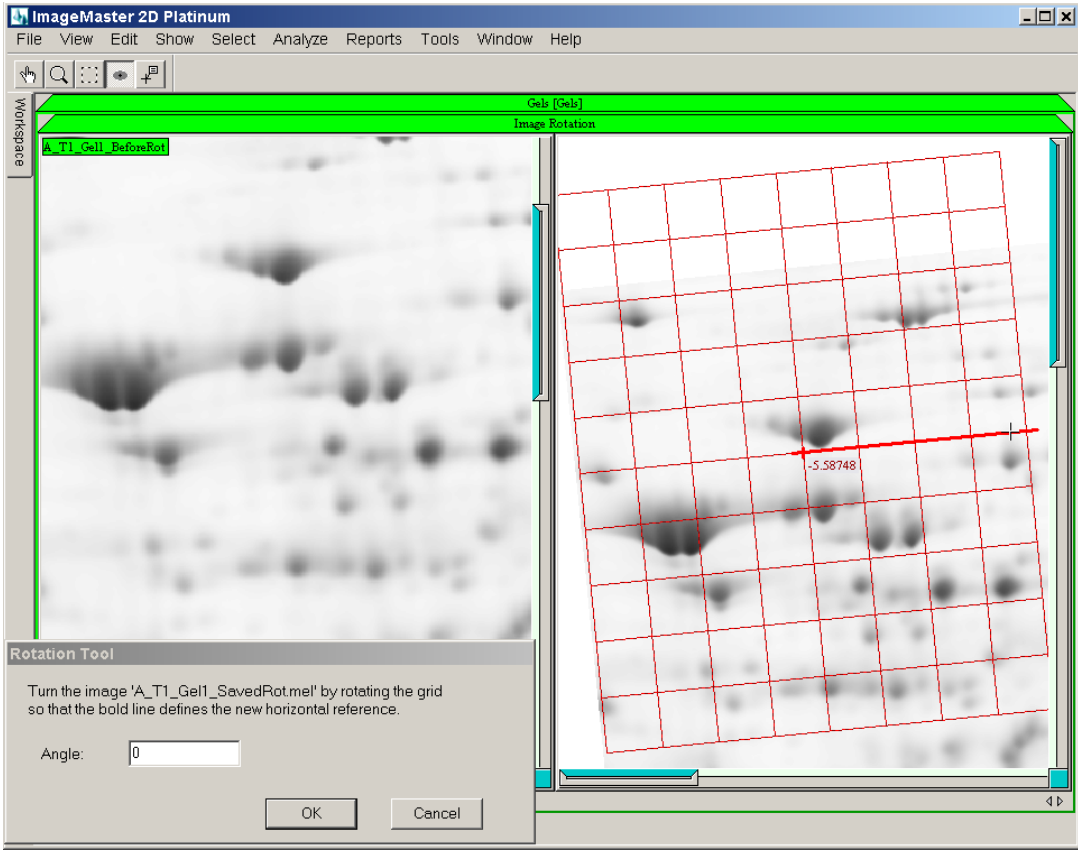


Figure 6-18. Rotation Tool. The grid is rotated until its bold line is parallel with what should be the new horizontal reference. When the mouse button is released, the gel image is rotated.

6.6.4 Flipping gels

Sometimes, images are scanned in the wrong direction, and you may have to flip them to get their correct mirror image. With ImageMaster, you can flip gels horizontally or vertically.

To flip your gels:

1 Select the gels to be flipped (in the same way) in a worksheet.

2 Choose *Tools* > *Gels* > *Flip* from the menu and select the desired option (*Horizontally* or *Vertically*).

3 Enter a new file name in the *Flipped Gels* box. Note that instead of changing each file name individually, you can add an extension, or replace the extensions of the original image files. You can also change the destination folder.

4 The flipped images are saved to the disk drive and opened in the ImageMaster window.

6.6.5 Scaling gels

ImageMaster lets you create smaller or larger copies of selected gels. This function is particularly useful for very large images where a reduction in size may significantly decrease the time and memory required for the analysis.

To reduce or increase the size of your images once they have been opened:

1 Select the gels to be scaled in a worksheet.

2 Choose *Tools* > *Gels* > *Scale* from the menu.

3 Enter the horizontal and vertical scale factors in the dialog box.

4 Give new file names or modify the file extensions.

5 Click *OK*. The scaled gels are saved on the hard disk and opened in the software. All spots and annotations are maintained.

6.6.6 Inverting gray levels

The last option in the *Tools* > *Gels* menu lets you change the gray levels of selected gels by inverting them. This means that if your image shows white spots on a black background, the inversion displays black spots on a white background (the required mode for analysis in ImageMaster).

To invert the gray levels of your gels:

1 Select the gels to be inverted in a worksheet.

2 Choose *Tools* > *Gels* > *Invert Gray Levels* from the menu.

3 Enter new file names in the *Invert Gels* box. Note that instead of changing each file name individually, you can add an extension, or replace the extensions of the original image files. You can also change the destination folder.

4 The inverted images are saved on the hard disk and opened in the ImageMaster window.

6.6.7 Cropping gels

With ImageMaster, you can crop your gels. That is, you can create new gels that only contain the defined regions of selected gels, together with the spots and annotations that were located in those regions.

To crop gels:

1 Define regions in selected gels using the *Region* tool. Delimit a particular area in each gel or use the Shift key to select the same region in all gels.

2 Choose *File* > *Save As* from the menu.

3 For each selected gel, enter the destination folder and file name. Click on *Save*.

4 ImageMaster asks you if you want to save only the selected area. Answer *Yes*.

5 New gels are created on the hard disk and inserted into the current project. The new gel images contain only the parts of the gels that lie within the specified regions. Any spots and annotations that were present in the saved region on the original gels are maintained.

You can also export and import a *Selection Box* to ensure that the final size of your cropped gels is identical, even between work sessions. A Selection box is a region with an anchor attached to it. You position the anchor on an easily recognizable protein spot, and as the region moves with the anchor, you will always crop a similar part of the gel.

To export a Selection Box:

1 With the *Region* tool, define a region on a gel that you would typically like to crop.

2 While holding the Alt key, click on a characteristic spot that can easily be found in all gel images. An anchor appears on the clicked position. Note that this anchor may be located inside or outside the gel region.

3 If you are not satisfied with the position of the anchor, you can redefine it by pressing Alt again while clicking on a new location. You can also remove the entire Selection Box by double clicking in the gel image.

4 Select the gel in which you defined the Selection Box.

5 Choose *File* > *Export* > *Selection Box* to save the Selection Box to a file with the extension .cpt (Crop Tool).

6 Enter a destination folder and file name. Click on *Save*.

To import a Selection Box:

1 Select the gels to be cropped using a Selection Box.

2 Choose *File* > *Import* > *Selection Box* and select the previously saved file. Click on *Open*.

3 The Selection Box appears on the selected images.

4 By clicking inside the box, with the *Region* tool, and dragging it, you can move the Selection Box to superimpose the anchor on the characteristic spot.

5 The gels can now be cropped as described above, by using *File* > *Save As*.

6.7 Reporting on gels

6.7.1 Gel report

The *Gel Report* (Figure 6-19) displays summarized information about the selected gels, such as image height and width in pixels (Rows and Columns), pixel dimensions of the scanned image (PixWidth and PixHeight), minimum and maximum gray levels before (MinGray and MaxGray) and after calibration (MinValue and MaxValue), as well as the intensity normalization parameters (Slope and Offset), the calibration function (Calibration) and calibration units (Unit). It also shows the Gel ID, the full file path of the image, the staining method, the number of detected or selected spots, the number of defined or selected annotations, the minimal and maximal values of pI and MW, the match set to which it belongs, and the class to which the gel was assigned.

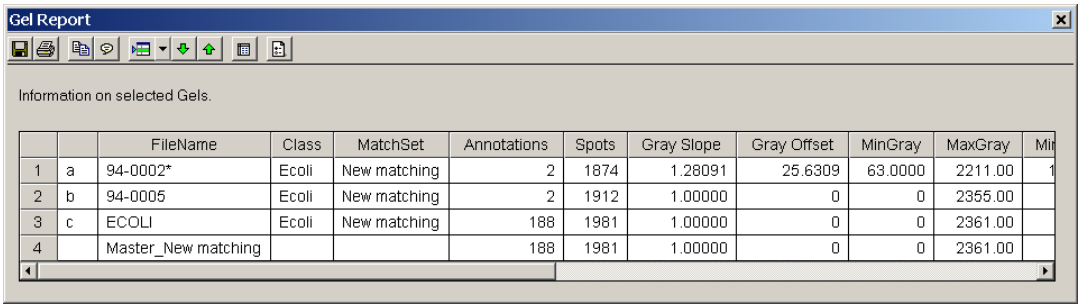
To display a Gel Report:

1 Select the gels that should be included in the report.

2 Choose *Reports* > *Gel Report* > *Current Template* in the menu.

3 The Gel Report is displayed.

4 Click on the *Settings* icon in the report toolbar to customize the report (see Chapter 5 for more details on report customization).



		FileName	Class	MatchSet	Annotations	Spots	Gray Slope	Gray Offset	MinGray	MaxGray	Mi
1	a	94-0002*	Ecoli	New matching	2	1874	1.28091	25.6309	63.0000	2211.00	1
2	b	94-0005	Ecoli	New matching	2	1912	1.00000	0	0	2355.00	
3	c	ECOLI	Ecoli	New matching	188	1981	1.00000	0	0	2361.00	
4		Master_New matching			188	1981	1.00000	0	0	2361.00	

Figure 6-19. Gel Report. The gel 94-0002 was normalized as revealed by the asterisk next to its name, and the values for Gray Slope and Gray Offset.

6.7.2 Gel description report

Besides the above information on selected gels, you can enter experimental data about your gel images, to be used for later reference by yourself or any colleagues. This information can include sample type, date of the experiment, operator name, pH range, SDS gel percentage, or staining. All this data can be entered in the dedicated *Gel Description Report* and stored in your gel files (Figure 6-20).

The Gel Description Report is available from the *Reports* menu. Initially, no descriptions are defined in the report, and only a column with the gel names is available.

To define descriptions for your gels:

1 Select the gels for which you want to add descriptions.

2 Choose *Reports* > *Gel Description Report* in the menu.

3 The Gel Description Report is displayed.

4 Click on the *Create Labels* icon in the *Gel Description Report* toolbar.

5 In the *Gel Descriptions* box, click the *Add* button.

6 In the *Add Category* box, enter the name of a new description category and click *OK*.

7 Set the **Category Attribute** to **Text**, **Number** or **Boolean**.

8 Continue creating new categories until you are finished.

9 To suppress an erroneous or unwanted category, select it from the **Categories** list and click the *Delete* button.

10 Click *OK* when you are finished.

The Gel Description Report now displays a column for each description category. You can directly add new information in the appropriate cells, or edit existing data. Just double click in a cell to start typing your information. When finished, a simple click in any cell ends the editing mode. The modified cells appear in dark green when the corresponding line is selected, or in gray when not selected (Figure 6-20). An asterisk in the report's window title reflects the fact that changes were made to it.

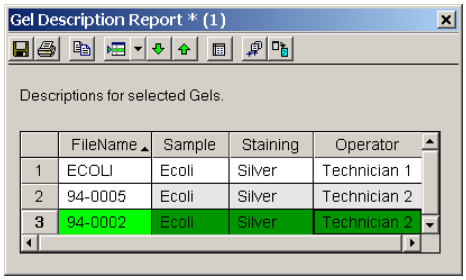


Figure 6-20. Gel Description Report. Gray or dark green cells were modified and need to be updated on the gel.

Subsequently, the *Update Gel* icon should be used to make the modifications definitive and to propagate the information to your gels. Note that the updated cells return to their original color. If all information is updated, the asterisk next to the report title also disappears.

Please note that you can keep the list of gel description categories for later use. To do this, click the *Create Labels* icon in the Gel Description Report. In the *Gel Descriptions* dialog box, the following options are available:

- Click **Save** and type a file name to store the current gel description categories.
- Click **Load** and choose a file name to reload an existing category list.

NOTE! The gel descriptions defined via the Gels Description Report are only valid for the gels that were selected during the creation of the report. To make the list of gel description categories a template for a particular workspace, which should be used for any open gels, you should define the gel description categories via the *Gel Descriptions* tab of the *Options* window (go to *Tools* > *Options)*. The procedure is the same as that described above. The only difference is that the defined gel descriptions are automatically displayed in any Gels Description Report displayed.

Alternatively, you can define gel descriptions by selecting gels and choosing *Edit* > *Gels* > *Add Description* from the menu. You will be asked to choose a gel description category or to type a new one, and to enter the description content. This option is particularly useful when you want to add identical descriptions to a series of gels because you can create many descriptions at a time, instead of having to define each one individually.

Similarly, you can delete all gel descriptions of a certain category for the selected gels. Select the gels and choose *Edit* > *Gels* > *Delete Description*.

6.7.3 Gel calibration report

If your gels were calibrated, you can find related information in the *Gel Calibration Report* (Figure 6-21), which is available from the *Reports* menu. It displays the gel name, Gel ID and the full file path of the image, the calibration function, the name of the step tablet used, the name of the person that did the calibration, and the calibration date.

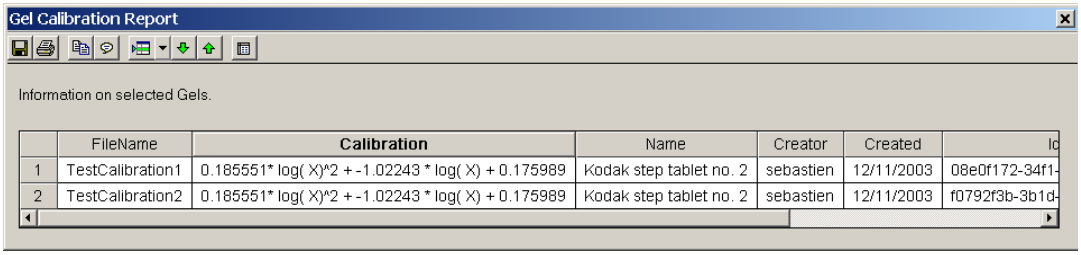


Figure 6-21. Gel Calibration Report.

6.8 Saving, exporting or printing images

6.8.1 Saving gels

In order to be stored in the gel file and thus to be available for future work, any changes made to a gel have to be saved before exiting the program or closing the gel. Regularly saving your gels enables you to recover from mistakes (although the multiple undo function allows you to return to previous analysis states) or to generate gel files with different names. Modifications are saved in the ImageMaster 2D Platinum file format, unless otherwise specified by the user.

ImageMaster does not automatically save the changes you make to a gel. Instead, you can control what data should be saved and when. Nevertheless, when you close a gel or leave the software, ImageMaster alerts you that modifications were made and gives you the possibility to save them.

NOTE! Please note that the *Save* and *Save As* functionalities in the *File* menu apply to gel data, in contrast to the *Save* feature in the Workspace, which pertains to the Workspace and Projects, and also saves the software options. Saving your gels and saving modifications to the workspace (see Chapter 4) are therefore two distinct operations.

The software also allows you to make regular backups of your work, that is, of the workspace with all the gel images and related files, and to restore this data when necessary. Find more details about this in Section 4.11.

Two options are available from the *File* > *Save* menu.

- **Worksheet** allows you to save all the changes made to the gels opened in the active worksheet, without giving details or asking for confirmation.
- **Save All** allows you to save the changes made to all open gels in all the worksheets. In this case, confirmation messages give the detail of the changes made and you have the choice to save them or not.

To save changes made to your gels:

1 Select the gels for which you would like to save changes.

2 Choose *File* > *Save* > *Worksheet* or *Save All* in the menu.

You may want to save multiple copies of gels or create files of defined regions by using the *Save As* command. This is useful when you do not want to overwrite the previous version of the gel in order to keep track of your analysis, or to return to a prior stage. Also use this command to crop your gels or to save files in earlier formats (Melanie II or Melanie 3).

To save gels with a different name or format:

1 Select the gels to be saved.

2 Choose *File* > *Save As* from the menu.

3 For each selected gel, enter the destination folder and file name, and pick the desired file format from the **Save as type** list.

To save gel regions:

1 Select regions in the pertinent gels. Define a particular region in each gel or use the Shift key to select the same region in all open gels.

2 Select the gels for which a cropped region should be saved.

3 Choose *File* > *Save As* from the menu.

4 For each selected gel, enter the destination folder and file name, and pick the desired file format from the **Save as type** list. Click on *Save*.

5 ImageMaster asks you if it should save only the selected area. Answer *Yes*.

6.8.2 Exporting gels and windows

To files

Rather than saving your gel images in the ImageMaster file format, you may want to export them to a different file format (TIFF, BMP or PNG). In this case, the gel images are exported as 8-bit, flat, rasterized images without any structure. This means that gel components such as spots and annotations are saved exactly as they appear on the screen, but are no longer recognizable as ImageMaster objects and therefore become part of the image. Consequently, exported gel images should only be used for presentation purposes and not for further analysis with any software package.

To export gels or selected gel regions:

1 If you want to export gel regions, start by defining them with the *Region* tool. Define a particular region in each gel or use the Shift key to select the same region in all open gels.

2 Select the gels that you would like to export or the gels for which you would like to export a selected region.

3 Choose *File* > *Export* > *Image to File* from the menu.

4 For each selected gel, enter the destination folder, file name and file type in the *Export Image As* dialog box.

5 If a region was defined, ImageMaster asks you whether you want to export only the selected area. Answer *Yes* or *No*.

You also may want to export a view of the active worksheet.

To export a worksheet:

1 Choose *File* > *Export* > *Worksheet to File* from the menu.

2 Enter the desired folder, file name and file type in the *Export Image As* dialog box.

To the clipboard
Alternatively, you can export images to the clipboard for direct pasting into another software. The procedure is very similar to exporting images to files. However, you can only export one gel image at a time to the clipboard.

To export a gel or a selected gel region:

1 If you want to export a gel region, first outline it with the *Region* tool.

2 Select the gel to export.

3 Choose *File* > *Export* > *Image to Clipboard* from the menu.

4 If no region was selected, the entire gel image is sent to the clipboard. Otherwise, only the region is exported.

5 You can then paste the image into the preferred software.

To export a view of the worksheet to the clipboard:

1 Choose *File* > *Export* > *Worksheet to Clipboard* from the menu.

2 You can then paste the image of the worksheet into the preferred software.

6.8.3 Printing gels

ImageMaster provides various printing options. Print selected gels or regions of gels, one image per page, or even the active worksheet. Whatever your choice, the image is printed as it is displayed on the screen retaining objects and properties such as spots, annotations, contrast mapping and pseudo colors, alignment, zoom, grid, etc.

NOTE! With a zoom factor of 1, the printed gel image takes the full paper width. You can adapt the zoom factor to decrease the size of the printed image.

To print gels or selected gel regions:

1 To print only regions, start by drawing them with the *Region* tool. Define a cropped region in each gel or use the Shift key to select the same region in all open gels.

2 Select the gels that you would like to print.

3 Choose *File* > *Print* > *Images* from the menu.

4 If a region was selected, ImageMaster asks whether you want to print only the selected area. Answer *Yes* or *No*.

5 The selected gels or gel regions are printed.

To print the active worksheet:

1 Choose *File* > *Print* > *Worksheet* in the menu.

2 The image of the selected worksheet is sent to the printer.

You can change printing parameters such as printer name, paper size, paper orientation, etc. To do so, choose *File* > *Print* > *Page Setup* from the menu. This command calls the standard print window where printer-related settings can be modified.

7 Spots

7.1 Introduction

The elementary component of a gel is the Spot, which delineates a relatively tiny region in the gel where protein is present. This shape is automatically differentiated by a spot detection algorithm. Each spot in an image is assigned a unique number when the software identifies it, called the Spot ID. Moreover, a spot is quantified; its intensity, area and volume are computed.

The ultimate aim of defining spots is to compare protein expression changes between different gels. Therefore, two major requirements must be met:

- First, automatic spot detection needs to identify a maximum number of proteins while minimizing the number of artifacts incorrectly detected as spots and especially reducing the number of undetected proteins. This requires finding a good compromise between the sensitivity and the specificity of the spot detection algorithm. If the automatic spot detection results are not fully satisfactory, manual spot editing can be attempted (for non-DIGE gels).

- A second prerequisite is that the differential expression values are accurate and reproducible. The goal is not to measure the absolute protein abundance of each spot in an image. Within a gel, there is no quantitative relationship between the measurable amounts of protein in different spots. This is because proteins are colored with dyes that bind differently to different proteins. What is important, however, is that the relative quantities of the same protein spots in different gels are correctly evaluated. The spot detection and quantification methods in ImageMaster were specifically designed for this purpose. You will learn more on how this is done in the following pages.

In ImageMaster 2D Platinum 6.0, two different spot detection algorithms are implemented. The Melanie/ImageMaster algorithm is used for non-DIGE gel images. DIGE images are co-detected using the algorithm created by the DeCyder software development team.

7.2 Spot ID

Each spot in a gel has a unique identifier, called the Spot ID. Spot IDs of deleted spots are not reused. ImageMaster attributes a new ID to each new spot. When a spot is split, the child spot for which the coordinates are closest to the parent spot keeps the existing spot ID, the other child spot gets a new ID. When two spots are merged, the resulting spot is attributed the ID of the initial spot that was closest to the new center of gravity.

Through the combination of Spot ID and Gel ID, every spot in any of your experiments is therefore uniquely identified, thus preventing any confusion. Be aware that when you edit a spot (making it smaller or bigger) after having generated a report, the spot values in the existing report are not updated and are therefore incorrect.

7.3 Detecting spots in non-DIGE gels

The Melanie/ImageMaster spot detection algorithm is optimized to give relevant biological results with minimum user interaction. Only a few easy to use parameters need to be defined in order to automatically locate spots in your image.

NOTE! When you look at a detected spot in the *3D View* (see Section 6.4.6), you will notice that the borders are not localized at the base of the spot, especially for intense spots. This is normal because the spot contours displayed effectively reflect the quantified parts of the spot, and they do not correspond to the 'whole' spot, which is difficult to define. The reason for this lies in the quantification methodology described in Section 7.3.3.

7.3.1 Procedure

To detect spots efficiently we recommend previewing the spot detection results on a few small image areas (drawn with the *Region* tool before or during spot parameter optimization) so that you can decide whether the parameters should be adjusted or not. Each change in one of the spot detection parameters is immediately reflected in the selected region (Figure 7-1) except if you turn the **Auto Preview** option off in the *Detect Spots* window. In this case you need to click the *Preview* button to refresh the spot detection preview. The advantage of manual previewing is that it is quicker to make simultaneous changes to more than one parameter. Once you find the optimal parameters, you can detect all spots on the selected images.

Please note that you can already view certain properties of spots (for example, **Intensity**, **Volume** or **Saliency**) in the preview mode while the spots are not yet detected permanently. Do this by displaying the Cursor Information window (see Section 7.9) and moving your cursor over a spot or by selecting a spot and displaying a Spot Report (see Section 7.9). This is particularly useful for entering a valid Saliency parameter (see Section 7.3.2). Obviously, the *Detect Spots* window must remain open while doing this.

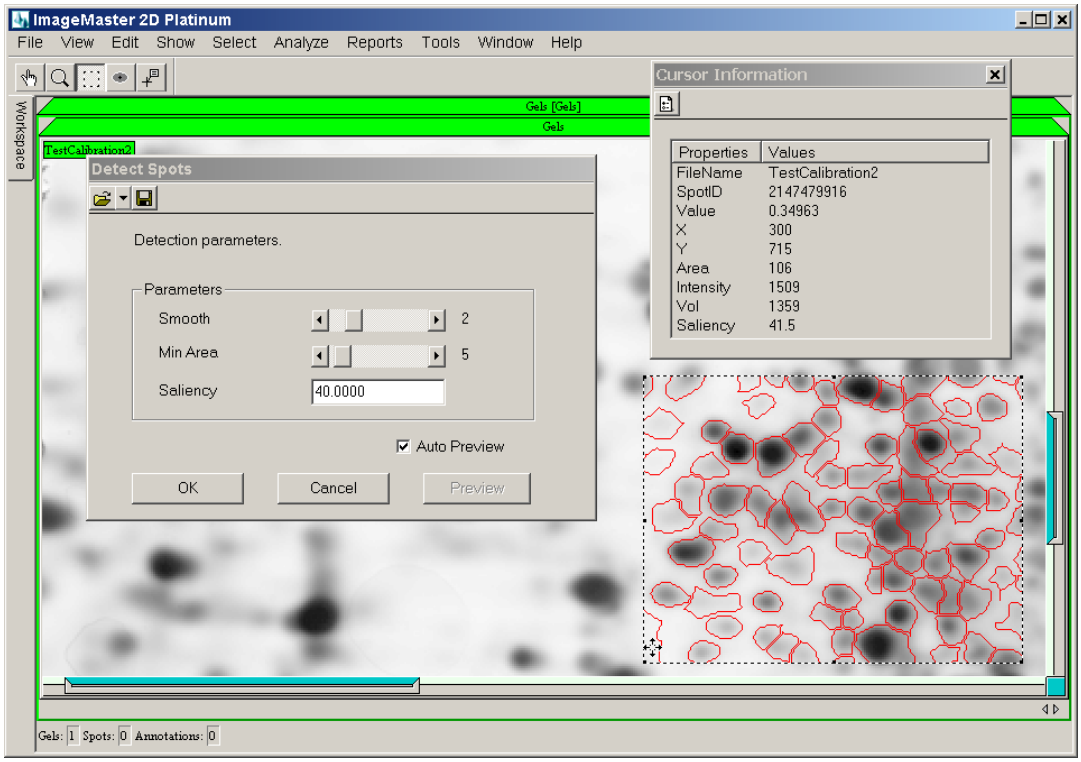


Figure 7-1. Adjusting spot detection parameters in real time.

To detect spots automatically:

1 Select the gel images for spot detection.

2 If necessary, choose *Show* > *Spots* > *Shape* > *Outlined* to see the spot borders more easily.

3 Click on the *Region* tool. Draw a rectangle around an area with representative spots on one or more of the selected gels. Note that you can still draw, resize or move regions while setting the detection parameters.

4 Choose *Edit* > *Spots* > *Detect* in the menu.

5 The *Detect Spots* window appears on the screen and the spots in the drawn regions of the selected images are detected with the default parameters. If you do not want the program to recalculate the spots in the preview regions for each parameter change, turn the **Auto Preview**

option off. To manually refresh the preview regions, simply click the *Preview* button.

6 Adjust the detection parameters. In particular optimize the **Smooth** parameter to detect all real spots and split the overlapping ones. Subsequently, filter out the noise by changing the **Saliency** and **Min Area** values. See below for more details on spot detection parameters.

7 When you are satisfied with the preview, click *OK* to detect all spots in the selected gels using the parameter values you have set. Please note that you can still change your gel selection at this point.

8 The spot shapes are displayed on the images.

You may want to save the current detection parameters so that they can be applied to other gels at a later time. To do this, click the *Save* icon in the toolbar of the *Detect Spots* window. Enter a file name and click *Save* in the *Spot Detection* dialog box. ImageMaster saves the detection parameters with the extension .dpm. By default, they are saved in the *ImageMaster\Menus* folder in the user's *My Documents* directory. Parameter sets saved in this default folder are always available from the *Open* icon in the toolbar of the *Detect Spots* window.

7.3.2 Spot detection parameters

Spot detection parameters are best adjusted in the following order:

- **Smooth**: First set the Smooth parameter. It fixes the number of times ImageMaster will smooth the image before detecting spots, using a smooth-by-diffusion algorithm. The Smooth parameter should be optimized to detect all real spots and split as many overlapping spots as possible without being concerned about noise spots (that can be filtered with the Saliency and Min Area parameters).

- **Saliency**: The Saliency parameter is a measure based on the spot curvature. It indicates how far a spot stands out with respect to its environment. Real spots generally have high saliency values whereas artifacts and background noise have small saliencies. Although the Saliency is an efficient quantity for filtering spots, it is also highly dependent on the images (for example, image resolution and depth). Some gels need a saliency value of 10 for correct filtering. Others may necessitate a value of 5000. To estimate the saliency range to use with your images, you can display a Cursor Information window or Spot Report and look at the saliency value given for a spot that you would like to suppress. Enter this value in the Saliency field of the Detect Spots window. The spot detection algorithm then discards all spots with saliencies smaller than the specified threshold.

- **Min Area**: After setting an appropriate Saliency to filter out all noise spots, there may still be noise in your gel that cannot be eliminated without suppressing real spots. This often happens with dust particles that consist of a few very dark pixels. Get rid of these artifacts by using the Min Area parameter. It eliminates spots that have an area smaller than the specified threshold (expressed in number of pixels).

7.3.3 Spot quantification

Once spots are detected, ImageMaster automatically computes the amount of protein present in each spot. Figure 7-2 illustrates the principles of spot quantification in the Melanie/ImageMaster algorithm. Measuring the protein quantification values in this way has the advantage of being more robust and reproducible when calculating protein expression variations (relative quantification).

- **Intensity**: The program first calculates the intensity of a spot. The intensity is based on the highest calibrated pixel intensities in the spot from which the background has been withdrawn. The background is defined as the minimum pixel value in the spot neighborhood.

- **Area**: The area of a spot is not determined at the spot base because the base is often arbitrary and difficult to determine. It is calculated at an intermediary height of the spot. More precisely, ImageMaster computes the area at 75% of the spot intensity, as measured from the peak of the spot. The spot outlines displayed in ImageMaster exactly encircle this computed spot area (expressed in mm^2).

- **Vol**: The volume of a spot is calculated as the volume above the spot outline, which is situated at 75% of the spot height (as measured from the peak of the spot). In Figure 7-2, the measured volume of the spot is hatched. Please note that the volume values, like the intensities, depend on pixel intensity calibration (see Section 6.5.1).

ImageMaster additionally calculates the relative intensity (%Intensity) and relative volume (%Vol) for each spot. These are normalized values that remain relatively independent of irrelevant variations between images. By definition:

$$\%\text{Intensity} = \frac{\text{Intensity}}{\sum_{S=1}^{n} \text{Intensity}_S} \times 100$$

where Intensity_S is the calibrated intensity of spot S in a gel containing n spots.

$$\%\text{Vol} = \frac{\text{Vol}}{\sum_{S=1}^{n} \text{Vol}_S} \times 100$$

where Vol_S is the volume of spot S in a gel containing n spots.

These measures take into account variations due to protein loading and staining, by considering the total intensity or volume over all the spots in the image. This means that in an image where, globally, the spots are darker than in another image, the majority of spot volumes is higher. However, the bulk of %Vol should be similar to those in the compared image, at least for gels with similar spot patterns.

Please note that although the %Vol is a rather efficient measure for evaluating protein expression differences between gels, the %Intensity is not as relevant to use.

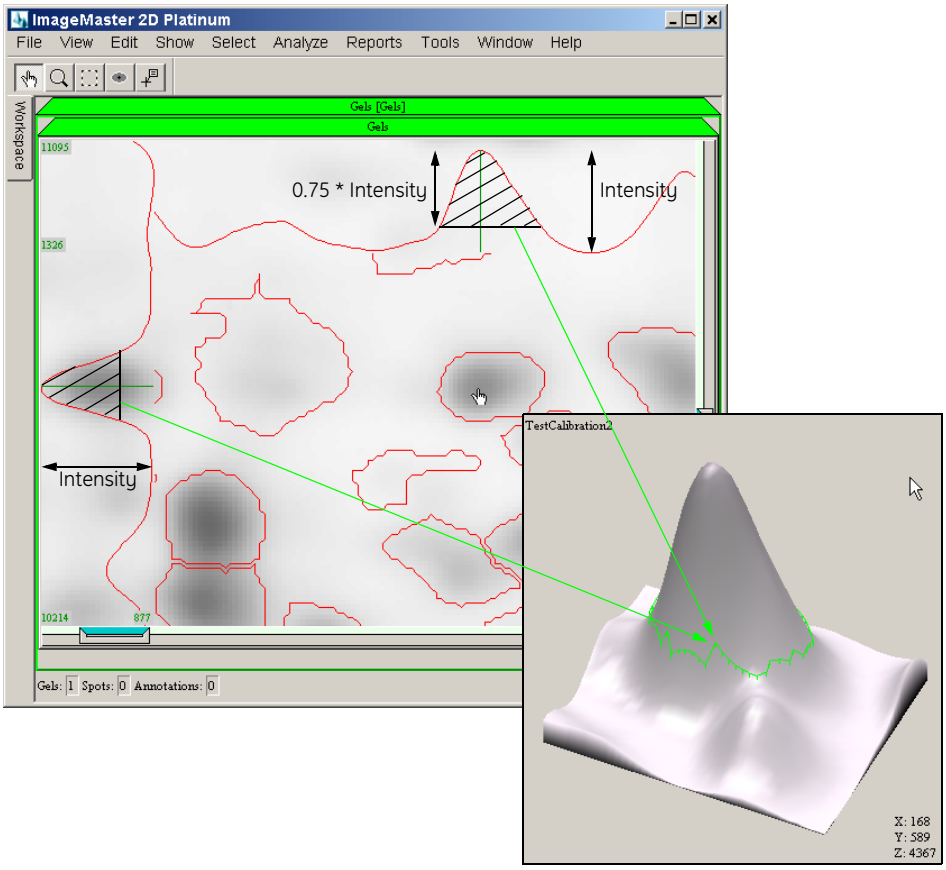


Figure 7-2. Spot quantification. The 3D View in ImageMaster reflects the spot shape and volume of what will effectively be quantified. The spot outline corresponds to the area at 75% of the spot height when measured from the peak.

When detecting spots, ImageMaster also computes the Saliency for each spot (see Section 7.3.2). It is displayed together with the above-mentioned quantification values in the Cursor Information window or a Spot Report.

7.4 Co-detecting spots in DIGE gels

The co-detection algorithm is designed to simultaneously process 1, 2 or 3 images derived from a single gel.

- Single detection - one image
- Double detection - two images
- Triple detection - three images

Single detection is performed on images of fluorescently post-stained gels used for picking, a case where there is a single image associated with the gel.

Double and triple detection takes advantage of the inherent comigration benefits of the CyDye DIGE Fluor dyes. A set of co-run images (2 images in double detection and 3 images in triple detection) are merged together thereby incorporating all spot features in a single image. Spot detection and spot boundary definition is then performed using pixel data from all the individual raw images and the merged image. The resultant spot map is overlaid back onto the original image files. Since the spot boundaries and the detection areas are identical for all images, the spots are effectively already matched. This process results in highly accurate volume ratio calculations.

7.4.1 Procedure

To perform spot detection on DIGE images:

1 Go to the *DIGE Gels* folder in the *Workspace* window.

2 Select the DIGE gels to be detected. Right click on one of the gels.

3 Choose *Detect* from the contextual menu.

4 The images corresponding to the selected gels are loaded and opened in a new worksheet.

5 In the *DIGE Spot Detection* window, enter an estimation (see below) of the **Number of Spots** present on the images. Click *OK*.

6 A status window appears showing the progress of the spot detection. Depending on your computer resources, the spot detection process can take some minutes.

7 The spots appear on the gels.

Alternatively, if your DIGE gels are already opened in the Display Zone, then you can select the gels and go to *Edit* > *Spots* > *Detect*. Enter an estimation for the **Number of Spots** to be detected in the *DIGE Spots Detection* window and click *OK*.

7.4.2 Spot detection parameter

When detecting DIGE images, you must enter an estimation of the **Number of Spots** present on the images. It is recommended that this value be overestimated to compensate for the detection of non-proteinaceous spots on the image, e.g. dust particles which can subsequently be excluded from the analysis using spot filtering (see Section 7.5.2 to learn more about spot filtering with the *Refine by Value* feature).

If all the spots have not been identified the spot detection process can be repeated with a higher number of estimated spots.

For example, for a mammalian lysate run on an 24 cm pH 4-7 Immobiline™ DryStrip and a large format gel, such as the Ettan DALT Gel (20 cm x 26 cm), a value of 2500 for the estimated Number of Spots should be satisfactory.

7.4.3 Spot quantification

Numerical data for individual spots are automatically calculated (e.g. volume, area, intensity, slope and volume ratio) and included in the DIGE Reports (see Section 7.10.3), Spot Reports (see Section 7.10.1) and Cursor Information window (see Section 7.9).

- **Vol:** Spot volumes (sum of pixel intensities within the spot boundary) are always expressed with background subtracted. Background is subtracted on a spot specific basis, by excluding the lowest 10th percentile pixel value on the spot boundary, from all other pixel values within the spot boundary. The spot volume is the summation of these corrected values.

- **Vol ratio:** Volume ratios (volume of first image spot/volume of second image spot) indicate the change in spot volume between two images.

NOTE! In the *DIGE Histogram* and *DIGE Report*, the first and second images are determined by the order in the workspace or the order in the worksheet. To obtain the inversed spot ratios, change the order of the gels in the workspace or the worksheet. In the *Spot Report* and *Cursor Information* window, the first image is the image mentioned in the FileName, the second image is the DIGE reference image.

NOTE! When using single detection the volume ratio value is 1.0 for all spots, since there is no second image.

7.5 Selecting spots

7.5.1 Spot tool

Spots can be selected with the *Spot* tool as long as they are visible on the image. Once selected, they are highlighted in green (Figure 7-3), unless the default spot colors were modified (see Section 7.6.2). If an annotation is attached to a spot, the annotation is also selected. Similarly, if you select an annotation or label with the *Annotation* tool, the linked spot is also selected.

To select a spot, make sure the *Spot* tool is activated and then click on the spot. To select more than one spot, select the first one, and then hold down the Shift key while clicking on additional spots.

To select all spots in a region, position the cursor at the top left position of the desired region, hold down the mouse button, and then drag the cursor to the bottom right position. All spots in the designated region will be selected and highlighted in green.

To deselect all spots, select the images in which you would like to deactivate all spots, and click in one gel (not on a spot).

7.5.2 Select menu

Additionally, you can select spots by using the items in the *Select* > *Spots* menu. Some of the following are particularly useful for filtering out artifacts (especially *Refine by Value*, possibly in combination with *Inverse Selection*):

- **By ID**: Allows the selection of a spot based on its Spot ID. Just enter the desired spot identifier in the *Select Spots by ID* dialog box.
- **By Color**: When a specific color is assigned to particular spots (using *Show* > *Spots* > *Set Color*), the spots can be selected by choosing the corresponding color from the submenu.
- **In Region**: This option allows you to select all spots in any regions that were drawn (with the *Region* tool) on selected images.
- **From Report File**: If any Spot Reports were previously saved, this function enables the reselection of all spots contained in the specified report file.
- **All**: Highlights all spots in the selected images.

- **Refine by Value**: This feature enables the selection of spots based on their spot value (Vol, Area, Intensity, %Vol or %Intensity), coordinates (X or Y), pI or MW. Depending on the detection algorithm used, spots can also be selected based on their Saliency, Volume Ratio or Slope. Make a selection of spots and then choose *Select* > *Spots* > *Refine by Value* from the menu. Choose the quantitative value you want to use for your refinement. Indicate the greater than (>=) and/or lower than (<=) limits of the interval by checking the appropriate boxes and entering the desired numbers. Spots whose values are included in the defined interval remain selected.

- **Inverse Selection**: If some spots are already selected, this option deactivates the selected spots and selects all the unselected ones, thus inverting the selection criteria.

7.5.3 Reports

You can select spots directly from an open Spot Report or DIGE Report. You can do this by double clicking on a spot in the report, or by selecting one or several spots and clicking the *Select on Gels* icon in the report toolbar. In this way, you can also select spots from an Intra-Class Report, as long as the spots belong to selected matches, or from reports on Labels, Annotations or Categories if the spots have linked annotations.

7.6 Displaying spots

7.6.1 Spot shape

Once spots are detected, you can display their shapes on the gels in four different ways (Figure 7-3), available from the *Show* > *Spots* > *Shape* menu:

- **Crossed**: Draws a cross in the center of gravity of each spot.

- **Outlined**: Displays the spot border.

- **Filled**: Shows the area of the spot.

- **Outlined/Filled**: Shows the spot border for inactive spots (Outlined mode) and the area of the spot for selected ones (Filled mode).

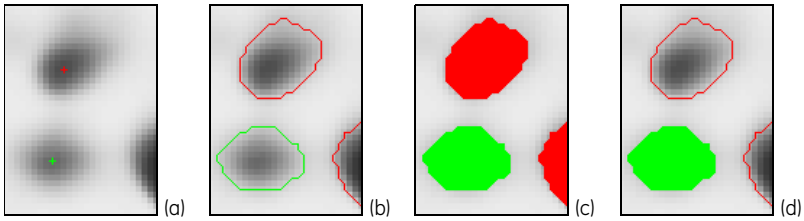


Figure 7-3. Possible spot shapes are (a) Crossed, (b) Outlined, (c) Filled, (d) Outlined/Filled. Selected spots appear as green crosses, green outlines or green surfaces.

The default spot shapes for newly opened gels can be fixed in the *Display* tab of the *Options* window (accessible by choosing *Tools* > *Options* in the menu).

7.6.2 Spot color

By default, unselected spots are displayed in red, selected spots in green and overlapped spots in blue. You can change these default colors in the *Display* tab of the *Options* window (accessible by choosing *Tools* > *Options* in the menu). Click on the colored box you wish to change and the *Color* dialog box opens. Choose the preferred color from the spectrum and click *OK*.

You can also change the color of a selection of spots as you go.

To change the color of selected spots:

1 Select the spots you want to change the color.

2 Choose *Show* > *Spots* > *Set Color* in the menu and pick one of the three proposed colors. Please note that the *Default* option corresponds to the spot color defined in the ImageMaster *Options* (accessible by choosing *Tools* > *Options* in the menu) .

3 ImageMaster displays the selected spots with the chosen color.

4 The colors remain displayed this way until you choose *Show* > *Spots* > *Set Color* > *Reset All* to revert back to the default spot colors.

7.6.3 Show / hide spots

You may want to hide some spots temporarily, for clarity or illustration purposes, or perhaps to concentrate only on those spots you consider important. Hiding spots also corresponds to filtering the spots because you cannot select hidden spots and they therefore do not appear in generated reports. Hiding spots has the additional effect of displaying images faster.

To show or hide particular spots, you can use one of the following options available in the *Show* > *Spots* menu:

- **Show All**: Makes all the spots on the selected images visible.
- **Hide All**: Hides all the spots on the selected images.
- **In Region**: Shows all spots in the region drawn with the *Region* tool. This option is handy when all spots were previously hidden.
- **Only Selected**: Shows only the selected spots and hides all others. This feature is helpful when performing a visual inspection of the spot detection or of the gel analysis results.
- **Hide Selected**: Hides the selected spots.

7.6.4 Show / hide spot IDs

You can find out the Spot IDs for specific spots via the Cursor Information window (see Section 7.9) or a Spot Report (see Section 7.9). However, sometimes you may want to display the identifiers of selected spots directly on the image.

To show or hide Spot IDs:

1 Select the spots you want to display the Spot ID for.

2 Choose *Show* > *Spots* > *Show ID* from the menu.

3 The Spot IDs for the selected spots are displayed close to each spot center.

4 You can hide all Spot IDs again by choosing *Show* > *Spots* > *Hide All ID*.

7.7 Adding / modifying spots

Quantitative protein data, and in particular the spot volume, are highly dependent on an optimal and reproducible definition of the spot borders and a correct splitting of partially overlapped spots. To guarantee reproducibility of quantitative work it is therefore recommended to create spots by using the automatic spot detection algorithm in ImageMaster and to avoid manual editing as much as possible. At most, spots should be manually separated where necessary. In spite of these words of warning, you still have the capabilities to create, modify or delete selected spots in ImageMaster.

NOTE! Spot editing is not allowed on aligned gels (because image warping may deform spots), and on DIGE gels. For edited spots, the Saliency value becomes zero.

7.7.1 Editing spots

In ImageMaster, spot editing is done directly on the image although one needs to be in the special spot-editing mode.

To modify spots:

1 Select the image you want to work on.

2 Choose *Show* > *Spots* > *Shape* > *Outlined* to see the spot borders more easily. It is also recommended to show any hidden spots (*Show* > *Spots* > *Show All*) in order to prevent the addition of already existing spots (and thus generating overlapped spots).

3 Choose *Edit* > *Spots* > *Edit Enabled* in the menu (a check mark next to the option indicates it is activated). The icon of the *Spot* tool in the main toolbar also changes to reflect the fact that spot editing is activated (Figure 7-4).

4 Select the spot you would like to edit with the *Spot* tool.

5 Carry out the desired spot modifications, as described below.

6 To deactivate this option, choose *Edit* > *Spots* > *Edit Enabled*. *Edit Enabled* is no longer checked in the menu.

(a) (b)

Figure 7-4. Spot tool when (a) spot editing is not activated and (b) spot editing is activated.

NOTE! You are still able to select spots while spot editing is activated. Nevertheless, we recommend you disable the *Edit Enabled* option whenever possible in order to prevent accidental modifications to your spots.

Increase the size of a spot

Outline the area that you would like to add by holding down the left mouse button while starting at one point on the selected spot and moving to another point within the spot (Figure 7-5).

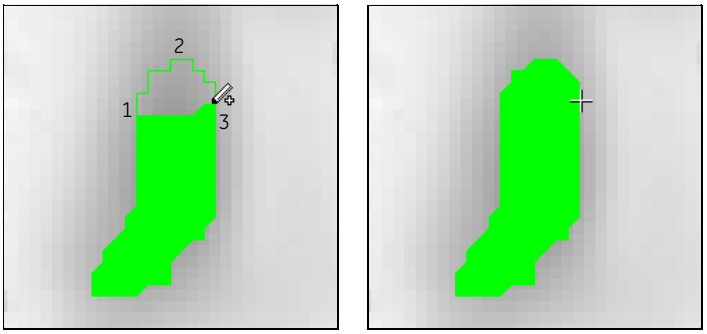


Figure 7-5. Increasing the size of a spot. The green line defines the portion of the spot that will be added (the numbers indicate the successive positions of the mouse cursor). The plus sign next to the pen indicates that one is in the 'adding' mode and the pen must start to draw from a point within the spot border.

Delete part of a spot

In order to erase part of a spot, you should also use the left mouse button, but now start from outside the initial spot outline and then partially encircle the area of the spot that you would like to suppress (Figure 7-6). Please note that the portion of the spot that contains the center of gravity in the initial spot will be conserved.

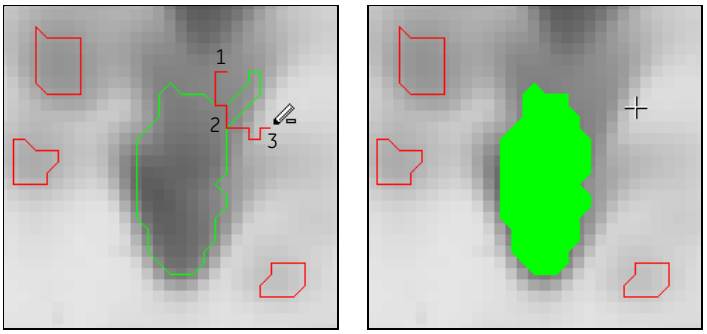


Figure 7-6. Deleting part of a spot. The red line outlines the portion of the spot that will be removed (the numbers indicate the successive positions of the mouse cursor). The minus sign next to the pen indicates that one is in the 'deleting' mode and the pen must start to draw from a point outside the spot border.

Split a spot

First, select the spot to split. Only one spot should be selected. Then right click outside the spot and, while holding down the right mouse button, draw a line through the spot at the position where it should be split (Figure 7-7).

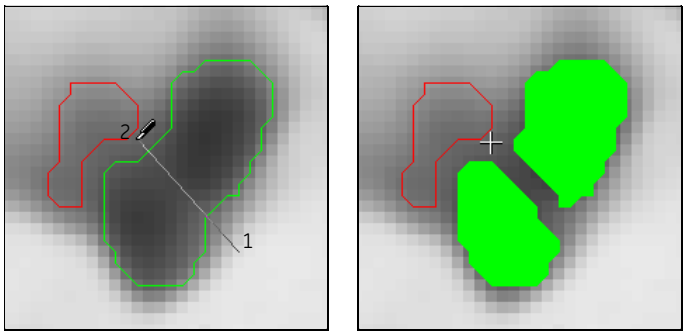


Figure 7-7. Splitting a spot. The grey line shows where the spot will be split (the numbers indicate the successive positions of the mouse cursor). The cutter indicates that one is in the 'splitting' mode.

Merge two spots

Select the first spot. While holding down the Shift key and the left mouse button, draw the fusion zone starting from within the second spot, going through the first one, and finishing within the second spot (Figure 7-8).

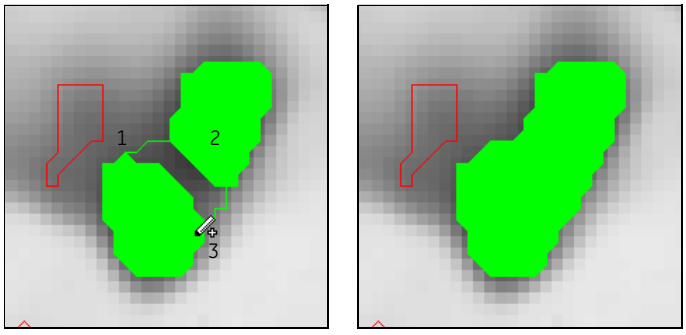


Figure 7-8. Merging a spot. The green trajectory defines the region where the two spots will be fused (the numbers indicate the successive positions of the mouse cursor). The plus sign next to the pen indicates that one is in the 'adding' mode.

Adding spots

When spot editing is enabled, the *Spot* tool can also be used to add a new spot to your gel.

To add a new spot to an image:

1 Choose *Edit* > *Spots* > *Edit Enabled* from the menu.

2 Click on the *Spot* tool.

3 Double click on the desired location in your image.

4 Use the slider to set the disc radius in the *Adding Spot* dialog box. The disc radius, expressed in number of pixels, determines the radius of the circular spot to be drawn.

5 A new spot (circle) with the specified radius is drawn at the desired location.

In addition, you can add spots to an image by copying them from other gels, provided the images were previously matched (see Chapter 9 for more details on matching). To do this perform the steps described below. Note that ImageMaster automatically creates matches between the selected spots and the newly created ones.

To copy spots from one matched image to another:

1 Select spots on a matched image.

2 Select one or more images you want to copy the spots to.

3 Choose *Edit* > *Spots* > *Copy From Image*.

4 The new spots are added to the selected images.

Once new spots are added to an image using either of these methods, you can make modifications to the newly created spot using the procedures described above.

Deleting spots
To remove spots from an image, just select the spots you want to delete and choose *Edit* > *Spots* > *Delete* from the menu.

7.8 MW and pI calibration

If you have a gel with pI/MW standards, ImageMaster can compute approximated pI and MW values for all the spots/pixels in this image, as well as any other images matched to it.

The principle is rather simple. You just have to define pI_MW annotations for a certain number of spots/pixels in the gel. The calculated pI and MW values for all spots in this gel and any matched gels automatically become available from a Spot Report, DIGE Report or the Cursor Information window (see below). In addition, pI and MW grid lines can be displayed over the images (see Section 6.3.5).

To define pI_MW annotations on an image:

1 Select the image for which you know the pI and MW values for several protein spots. Spots may or may not have already been detected in this image.

2 Click on the *Annotation* tool.

3 Double click on a spot (pixel) for which you know the pI and/or MW values.

4 Select the **pI_MW** category in the *Create Annotation by Click* window.

5 Enter the known pI and MW values, respectively, separated by a space. Replacing one of the values with -1 means that no value is set.

6 Do this for a sufficient number of protein spots that are distributed over the whole image area. Obviously, the more spots and annotations, the better the approximated pI and MW values will be.

ImageMaster does the following to calculate approximate pI and MW values: In the case of pI, it looks up the two closest annotations to the left and to the right of the spot for which the pI will be determined, and then interpolates between these 2 points. Since ImageMaster does not have any information about the experimental (possibly non-linear) pI scale, the calculated values are only approximate. In the case of the MW of the spots, the procedure is similar, except that ImageMaster searches for the closest spots above and below the spot for which the MW will be determined and it makes a logarithmic interpolation.

Extrapolating pI and MW values is more complicated. For instance, if the pI of a spot on the extreme right side of your gel is to be determined, the program looks for the two closest spots to the left of the spot in question. If these two spots are sufficiently distant from each other (in order to decrease the error), the value for the spot in question can then be extrapolated.

Normally, the pI and MW values in the Spot Report or Cursor Information window should be the same as in the defined pI_MW annotations. However, this is only the case if the annotations are attached to actual spots and not just to pixel positions in the image. If an annotation is attached to a pixel, the pI and MW values for the spot that lies closest to it will be slightly different from that of the pixel (to which the annotation is attached). You can solve this ambiguity by linking the annotation to the spot.

7.9 Cursor information

The *Cursor Information* window can be used at any time to display information on pixels (X and Y coordinates, pI and MW estimates or raw pixel values) and spots, if

spots were detected. Information on spots includes the name of the image on which the spots were detected, the Spot ID, the coordinates of the center of gravity (X and Y), and the quantification values (Intensity, Area, Vol, %Intensity and %Vol). Depending on the spot detection algorithm used, the Saliency, Vol Ratio and Slope will also be available.

NOTE! In the Cursor Information window, the Vol Ratio given corresponds to: Volume of the spot under the cursor/Volume of the spot on the DIGE reference image.

To display cursor information:

1 Choose *Window* > *Cursor Information* in the menu.

2 The *Cursor Information* window (Figure 7-9) appears on the screen.

3 Click on the *Settings* icon to define the data to be displayed in the window.

4 In the dialog box, select the attributes to be shown in the *Cursor Information* window from the **Hidden** list (using the Shift or Ctrl keys to make multiple selections), and transfer them to the **Visible** list using the right-pointing arrow button. Similarly, you can remove attributes from the *Cursor Information* window by selecting them in the **Visible** list and clicking on the left-pointing arrow button. You can also double click on an attribute to transfer it from one list to the other. Click *Apply*.

5 The selected attributes are displayed in the *Cursor Information* window.

6 Position your cursor over the pixel or spot of interest. The *Cursor Information* window displays the desired information. If the **Value** field shows **Unloaded**, the raw pixel values are unavailable. In this case, choose *Edit* > *Gels* > *Raw Image* > *Load* to load the image data for the gels.

The order of the items in the Visible attributes list determines the order of the items in the Cursor Information window. You can therefore adjust the display by adding your attributes in sequential order to the Visible attributes list.

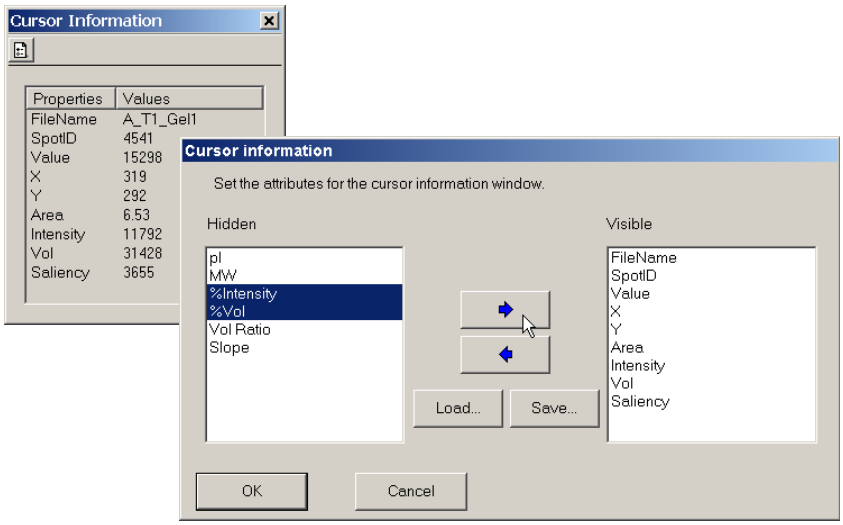


Figure 7-9. (a) Cursor Information window and (b) Cursor Information dialog box for specifying the Settings.

Please note that these settings can be made permanent by going to the *Cursor Information* tab in the *Options* (accessible by choosing *Tools* > *Options* in the menu). Your adapted settings can also be saved and used as templates. For this purpose, the following options are available from the *Cursor Information* dialog box:

- **Save**: Allows you to save the new Cursor Information template.
- **Load**: Opens an existing template file.

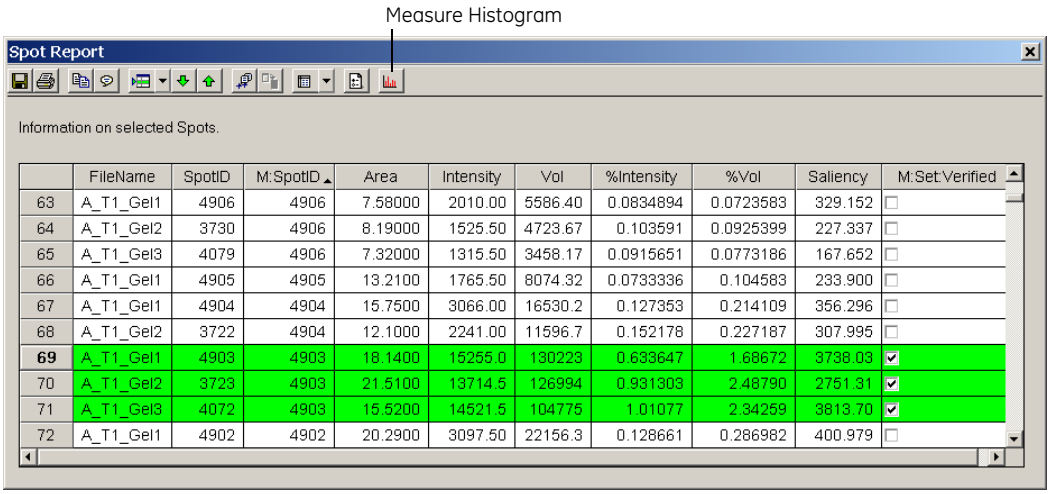
7.10 Reporting on spots

7.10.1 Spot Report

The *Spot Report* summarizes all relevant information about the selected spots: their identification number (Spot ID), the name of the image on which they were detected, the coordinates of their center of gravity (X and Y), the quantification values (Intensity, Area, Vol, %Intensity and %Vol), pI, MW and all linked labels. Depending on the spot detection algorithm used, the Saliency, Vol Ratio and Slope will be available. When working in a [MatchSets] or [Classes] worksheet, you can also display information on the corresponding spot in the master gel (Figure 7-10).

NOTE! When displaying a Spot Report for DIGE images, the Vol Ratio given corresponds to: Volume of the spot on the image mentioned in the FileName/ Volume of the spot on the DIGE reference image.

The Spot Report can be saved, printed, exported and used to sort through data. Please see Chapter 5 for more details on these functionalities (available as icons in a report toolbar) and the possibility of customizing the Spot Report.



	FileName	SpotID	M:SpotID	Area	Intensity	Vol	%Intensity	%Vol	Saliency	M:Set:Verified
63	A_T1_Gel1	4906	4906	7.58000	2010.00	5586.40	0.0834894	0.0723583	329.152	☐
64	A_T1_Gel2	3730	4906	8.19000	1525.50	4723.67	0.103591	0.0925399	227.337	☐
65	A_T1_Gel3	4079	4906	7.32000	1315.50	3458.17	0.0915651	0.0773186	167.652	☐
66	A_T1_Gel1	4905	4905	13.2100	1765.50	8074.32	0.0733336	0.104583	233.900	☐
67	A_T1_Gel1	4904	4904	15.7500	3066.00	16530.2	0.127353	0.214109	356.296	☐
68	A_T1_Gel2	3722	4904	12.1000	2241.00	11596.7	0.152178	0.227187	307.995	☐
69	A_T1_Gel1	4903	4903	18.1400	15255.0	130223	0.633647	1.68672	3738.03	☑
70	A_T1_Gel2	3723	4903	21.5100	13714.5	126994	0.931303	2.48790	2751.31	☑
71	A_T1_Gel3	4072	4903	15.5200	14521.5	104775	1.01077	2.34259	3813.70	☑
72	A_T1_Gel1	4902	4902	20.2900	3097.50	22156.3	0.128661	0.286982	400.979	☐

Figure 7-10. Report on selected spots in selected images.

Measure histogram

By clicking on a numerical column header in the Spot Report and then pressing the *Measure Histogram* icon, you can display a histogram showing the distribution of the values in the selected column (Figure 7-11). The purpose of such histogram is to graphically summarize the distribution of a univariate data set. It shows the following:

- Center (i.e., the location) of the data;
- Spread (i.e., the scale) of the data;
- Skewness of the data;
- Presence of outliers; and
- Presence of multiple modes in the data.

The *Measure* icon at the top of the *Measure Histogram* window allows you to choose between one of two options:

- The **Histogram** is obtained by splitting the range of the data into equal-sized bins (intervals). Then for each bin, the number of points from the data set that fall into each bin are counted. The horizontal axis represents the response variable .The frequency (i.e., counts for each bin) is displayed on the vertical axis.
- The **Cumulative Histogram** is a variation of the histogram in which the vertical axis gives not just the counts for a single bin, but rather gives the counts for that bin plus all bins for smaller values of the response variable.

Yellow guides move with your cursor and indicate, at the bottom of the window, the frequency (Y) and the response variable (X) for each bin. The red vertical line represents the mean value.

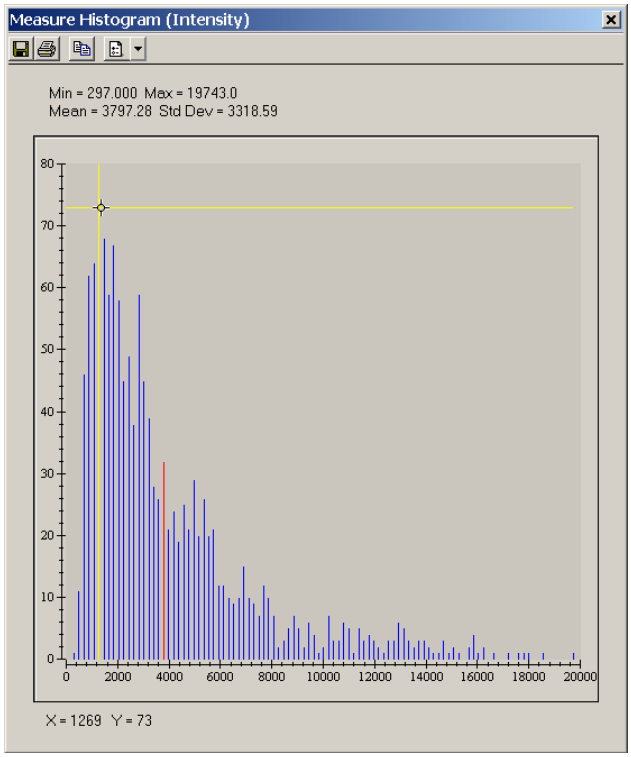


Figure 7-11. Measure Histogram.

7.10.2 DIGE histogram

A *DIGE Histogram* can be displayed when two co-detected images are selected (Figure 7-12). It shows data associated with detected spots in the selected images. Spot data is plotted against log volume ratio on the X-axis, using two Y-axes.

- The left Y-axis displays the spot frequency. The blue curve represents the frequency distribution of the log volume ratios.
- The right Y-axis represents the *Measure* parameter (see below) selected from the corresponding drop down menu in the toolbar of the DIGE Histogram window. A plotted single data point on the histogram represents an individual protein spot.

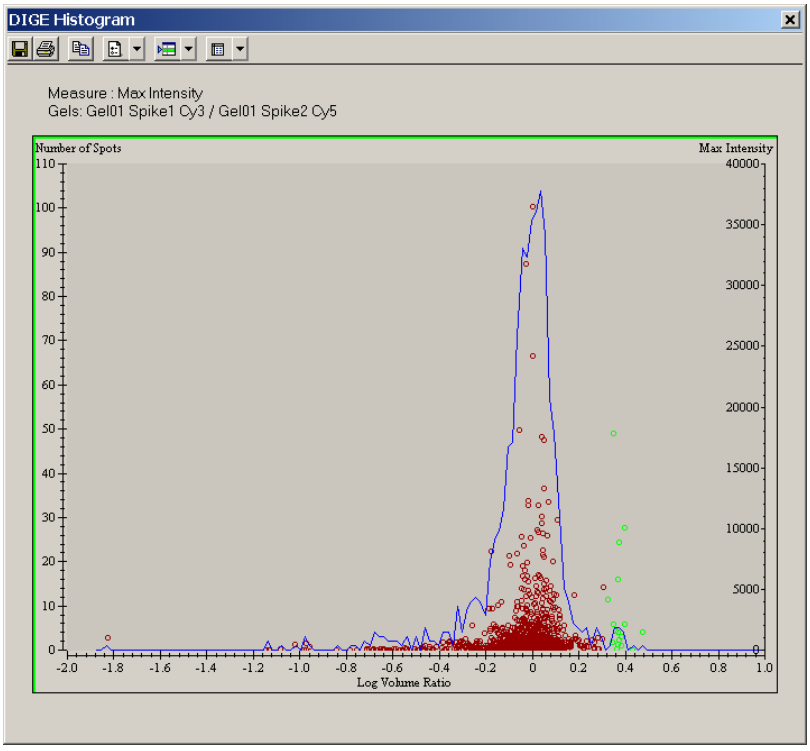


Figure 7-12. DIGE Histogram.

The DIGE Histogram is interactive. You can click on the data points representing spots and select them on the gels and any other open reports. Just select the spots by clicking on the corresponding points while using the Ctrl or Shift keys for multiple selections. To select all spots in a region, hold down the mouse button

and then drag the cursor to define an area. Use the options from the *Select on Gels* drop down menu in the toolbar to identify the corresponding spots on the gels and open reports.

The pull down menu next to the *Reports* icon in the toolbar can be used to display information related to the DIGE Histogram:

- The **Gel Report** option displays a Gel Report for the two selected gels.
- The **DIGE Report** item show the numerical values corresponding to the spots in the DIGE histogram (see below).

There are two ways of displaying a DIGE Histogram. Depending on which one you use, all detected spots or only selected spots are included.

To display a DIGE histogram containing all spots:

1 Select two co-detected images in the *DIGE Gels* folder of the workspace. Right click on one of the images.

2 Choose *Histogram* from the contextual menu.

3 A DIGE Histogram containing all spots from the selected gels is displayed.

To display a DIGE histogram containing selected spots:

1 Select two co-detected images in a [Gels] or [Classes] worksheet.

2 Select spots. Use the Shift or Ctrl keys to make multiple selections.

3 Choose *Analyze* > *DIGE* > *Histogram* in the menu.

4 A *DIGE Histogram* containing only the selected spots is displayed.

NOTE! In the DIGE Histogram, the Volume Ratio (used to calculate the log Volume Ratio on the X-axis) corresponds to: Volume of the spot on the first selected image/Volume of the spot on the second selected image. To obtain the inversed spot ratios, change the order of the gels in the worksheet.

Measure parameter

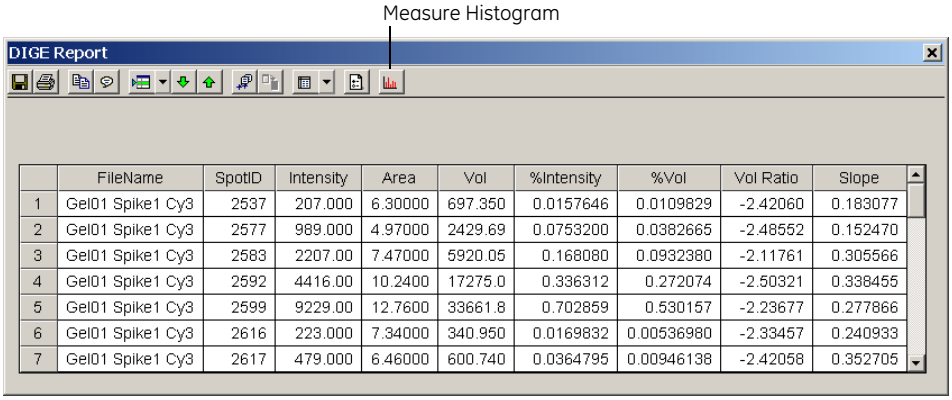
- **Max Slope**: Largest gradient associated with co-detected spots.
- **Area**: Number of pixels within the spot boundary.
- **Max Intensity**: Largest pixel value associated with co-detected spots.
- **Max Volume**: Volume of the largest of the co-detected spots.

7.10.3 DIGE report

A *DIGE report* is a special case of a spot report (see Section 7.10.1) enabling you to display NORMALIZED Vol Ratios (see the note below) for specific combinations of DIGE images (not only ratios compared to the DIGE reference image). Two images must be selected to generate a DIGE report. For each Spot ID in the report, generally two lines will show, one for each gel (Figure 7-13).

NOTE! The reported Vol Ratios in the DIGE report are NORMALIZED, so that the modal peak of volume ratios is zero (since the majority of proteins are not up or down regulated). This means that the Vol Ratio is expressed in the range of 1 to 1 000 000 for increases in spot volumes and –1 to – 1 000 000 for decreases in spot volumes. Values between –1 and 1 are not represented, hence a two-fold increase and decrease is represented by 2 and –2, respectively (and not 2 and 0.5 as might have been guessed).

NOTE! In the DIGE Report, the Vol Ratio shown corresponds to: Volume of the spot on the image mentioned in the FileName/Volume of the spot on the other selected image.



	FileName	SpotID	Intensity	Area	Vol	%Intensity	%Vol	Vol Ratio	Slope
1	Gel01 Spike1 Cy3	2537	207.000	6.30000	697.350	0.0157646	0.0109829	-2.42060	0.183077
2	Gel01 Spike1 Cy3	2577	989.000	4.97000	2429.69	0.0753200	0.0382665	-2.48552	0.152470
3	Gel01 Spike1 Cy3	2583	2207.00	7.47000	5920.05	0.168080	0.0932380	-2.11761	0.305566
4	Gel01 Spike1 Cy3	2592	4416.00	10.2400	17275.0	0.336312	0.272074	-2.50321	0.338455
5	Gel01 Spike1 Cy3	2599	9229.00	12.7600	33661.8	0.702859	0.530157	-2.23677	0.277866
6	Gel01 Spike1 Cy3	2616	223.000	7.34000	340.950	0.0169832	0.00536980	-2.33457	0.240933
7	Gel01 Spike1 Cy3	2617	479.000	6.46000	600.740	0.0364795	0.00946138	-2.42058	0.352705

Figure 7-13. DIGE Report

Depending on how you generate the DIGE Report, all the detected spots or only selected spots appear in the report, as it is the case for DIGE Histograms (see Section 7.10.2). If you display a DIGE Histogram from the contextual menu in the workspace, and then select *DIGE Report* from the *Reports* icon in the DIGE Histogram tool bar, all spots will be included. To include only selected spots, select spots in two gels and choose *Analyze* > *DIGE* > *Report*.

8 Annotations

8.1 Introduction

Individual pixels and spots in a gel image can be labeled with annotations. The annotations are used in functionalities such as alignment, matching, or flagging spots with their particular characteristics (protein name, database accession number and so on). Annotations can also be used to mark spots with common characteristics. Finally, annotations offer the possibility of linking and associating gel objects to external query engines or data sources of any format (text, html, spreadsheet, multimedia, database) located locally or on the Internet.

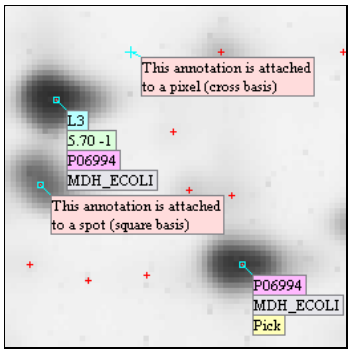


Figure 8-1. Annotations are composed of an annotation basis (square or cross), an annotation flagpole and a set of labels (flag).

An annotation is defined by its position on the gel (X and Y coordinates) and its set of labels. Each label belongs to a predefined or user-defined category. The label contents can be any textual data, numerical values or a Boolean value. The user can also decide that a label within a given category is unique.

As shown in Figure 8-1, each annotation is composed of a basis, which can be a square or a cross depending on whether the annotation is attached to a spot or a pixel, a flagpole (by default displayed in cyan) and a flag that consists of a set of colored labels.

8.2 Predefined label categories

Some predefined label categories are provided with the ImageMaster software. To help you visually identify the various label categories, they are automatically created using different background colors, as indicated below, after each category name:

- **Ac** (pink): This category is provided to hold the protein's accession number (AC) taken from a user-defined database, and can be the link to ImageMaster's remote database query engine. When such a link is defined, double clicking on a label of this category displays the corresponding protein entry in the selected database with the default Internet browser (see Section 8.3.1).

- **Landmark** (blue): This category is used to mark pixels or spots in the gels as reference points for the purpose of gel alignment or matching and for the calculation of corresponding locations between gels. Two annotations are considered as referring to the same point in different gels when they bear identical labels (of the same category).

- **pI_MW** (green): This category contains the known isoelectric point (pI) and molecular weight (MW) information, which is subsequently used to compute approximate pI and MW values for any point in a gel. You should enter the pI value first and MW value second, separated by a space. By replacing one of the values by -1, you indicate to the program that no value is set (probably because you do not know it).

- **Comment** (pale pink): This category is an example of a general category where users may store their comments. User comments can also be entered in any other user-defined category (see Section 8.3.1).

- **Set** (yellow): This is a generic category used to mark spots having common characteristics. All labels from a Set category share the same text, or in other words the same set name. Figure 8-1 exemplifies a set called Pick containing spots that need to be exported to a spot excision robot. See Section 8.3.2 for more details.

Please note that the default colors for the different label categories can be changed. You can do this by going to the *Categories* tab in the ImageMaster *Options* (accessible by choosing *Tools* > *Options* in the menu).

8.3 Creating annotations and labels

You can create two types of annotations. The first one, visualized with a cross basis, is simply connected to a pixel and has the same coordinates as that pixel. The second type of annotation concerns those that are linked to a spot. These annotations are marked by a square at their basis, and have the same coordinates as the spot to which they are linked, that is, the spot's center of gravity. In addition, the annotations are automatically selected when the linked spot is selected, and vice versa. (Figure 8-1)

NOTE! To create a spot-linked annotation, just click somewhere within a spot during creation (see below). To decrease the chance of missing a spot when clicking on the gel, you can display spots with the Outlined shape, rather than the Crossed option.

To create an annotation:

1 Click on the *Annotation* tool in the ImageMaster toolbar.

2 Double click on the pixel or spot in the gel where the annotation should be located.

3 In the *Create Annotation by Click* box, enter the name of a new category or choose one of the existing categories by clicking on its name.

4 When a new category is created, the *Create Category* box appears. Please see Section 8.3.1 to find out more about the parameters requested.

5 Type the desired label in the next dialog box, and click *OK* when finished.

6 The annotation is created and its label is displayed on the gel.

Labels can be added to an existing annotation. In fact, one annotation may have many labels, although it can only contain one label from each category. If one spot contains several proteins, it may need to carry different labels from the same category. You can do this by linking additional annotations to the spot.

To add a label to an existing annotation:

1 Click on the *Annotation* tool in the toolbar.

2 Double click at the basis of the annotation to which your label should be added.

3 In the *Create Annotation by Click* box, enter the name of a new category or choose one of the existing categories by clicking on its name.

4 When a new category is created, the *Create Category* window will appear. Please see Section 8.3.1 to find out more about the parameters requested.

5 Type the content of the label in the next dialog box and click *OK*.

6 The label is added to the existing annotation.

NOTE! Alternatively, you can add labels by selecting annotations or spots and choosing *Edit* > *Annotations* > *Add Label* in the menu. This option is particularly useful when you want to add identical labels to a series of spots or annotations because you can create many labels at a time, instead of having to define each one individually.

At any time, ImageMaster allows you to link an annotation to a spot, or do the opposite. This is helpful because sometimes you may miss a spot to which you wanted to link a newly created annotation or you may have decided that an annotation should not be linked to a given spot.

To link an annotation with a spot, click on the annotation basis and drag it to a spot. If, for some reason, an annotation already exists within a spot but is not yet linked, you can also select the annotation and choose *Edit* > *Annotations* > *Link with Spot*.

To unlink an annotation from a spot, select the annotation and drag it outside the spot or choose *Edit* > *Annotations* > *Unlink from Spot*.

8.3.1 Creating label categories

As mentioned above, ImageMaster offers a series of predefined categories. However, you can also create your own categories, which are always displayed with gray backgrounds. You can easily do this by adding an annotation using the procedure described above and typing the name of a new category. You must enter the constraints for the new category in the *Create Category* box (Figure 8-2).

Please note that the category constraints can also be modified at a later stage by going to the *Categories* tab in the ImageMaster *Options* (accessible by choosing *Tools* > *Options* in the menu). However, it is better to set the constraints while creating the category, otherwise you may have to adapt the existing labels to make them fit the new restraints.

Note that user-defined categories are only available from the category list as long as there is at least one label of this category in the open gels.

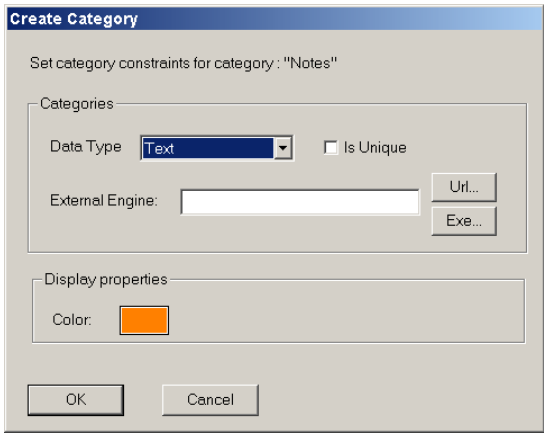


Figure 8-2. Create Category dialog box.

Data type

By default, the labels in ImageMaster can contain any character. However, to ensure coherent annotation data, the label contents can be constrained to only numerical values or Boolean entities. This is done by fixing the **Data Type** to one of the following:

- **Text**: Can contain any character.
- **Number**: Can only contain numerical values.
- **Boolean**: Can only take the values 0 and 1.

Notice that categories using the Boolean Data Type are displayed in the form of check boxes in most reports (except for the Label and Category Reports). Editing these labels corresponds to checking the box to indicate whether the item should take the value 0 (empty check box) or 1 (checked box).

Label categories of the Boolean Data Type are useful to indicate that you checked your data for specific properties, and that, based on these properties, you decide to classify your data points into two populations.

Imagine that you found 50 interesting proteins (significant expression variations) from a preliminary differential analysis. You can add a label of the Boolean type to each of these 50 protein spots and designate whether yes (value 1) or no (value 0) you want to export them to a spot excision robot. Thus, you can easily select spots to be picked, but you also have a record of which protein spots were carefully scrutinized.

Is unique

When you check the **Is Unique** box, you indicate to ImageMaster that each label on a gel within the new category should be unique. That is, no identical labels can be created. This is the case for the Landmark category, for instance. In order to prevent confusion as to which spots correspond in different gels, there should only be one spot marked with a particular label in each of the gels.

When the Is Unique box is checked, ImageMaster will not accept your label when an identical one already exists. You are asked to enter a new label. This is always the case for labels of the Landmark category.

External engine

The **External Engine** field should be used when you want to send HTTP queries to a CGI script on a server. A CGI (Common Gateway Interface) script is a program or script file executed on a Web server in response to a user request. It allows, for example, people from all over the world to query a database that is connected to the World Wide Web. The CGI script transmits information (such as a database accession number or object identifier) from the client to a database engine, receives back the results, and displays them to the client.

Virtually all databases on the Web, and in particular those containing 2-DE and other protein data (see Chapter 11), use CGI scripts to enable data queries. ImageMaster takes advantage of this fact by offering the possibility to link spots on gel images to protein data in 2-DE or other databases. All you have to do is input the appropriate query format (database address and query engine) in the External Engine field of the new label category and enter valid database accession numbers (AC) as labels.

When you subsequently double click on a label of such a category, ImageMaster opens your default Internet browser and launches an HTTP query that takes the form of a Web page address.

http://www.expasy.org/cgi-bin/nice2dpage.pl?P02649 The elements are:

- http://www.expasy.org/ (database **HTTP address**).
- cgi-bin/nice2dpage.pl? (database **query engine**).
- P02649 (database **accession number**).

This example relates to a query of the SWISS-2DPAGE database on the ExPASy Proteomics Server, using the nice2dpage query engine. As a result, the entry for Human Apo E, corresponding to the protein with accession number P02649, is returned in your browser.

To set the database HTTP address and query engine:

1 In the *Create Category* box, click on the *URL* button located next to the **External Engine** field.

2 The page http://www.expasy.org/ch2d/2d-access.html, detailing a list of federated 2-D PAGE databases and servers is displayed. This list provides the required database query formats (address and query engine).

3 Copy the desired database address and query engine. The necessary information generally ends with a question mark (?) or equal sign (=). If you would like to use the SWISS-2DPAGE, for instance, with the basic query engine, you should copy the sequence http://www.expasy.org/cgi-bin/get-ch2d-entry?.

4 Paste the copied information into the **External Engine** field.

To link to a database that is not federated or that does not contain 2-DE data:

1 Directly query the database you would like to link your spots to, until you find a specific protein (or other) entry. For example, you might display the entry for the protein structure 1BMT in the well-known Protein Data Bank (PDB).

2 Check in your browser whether you can delete parts of the Web page address without losing the database entry. In the example of the PDB, the original address http://www.rcsb.org/pdb/cgi/explore.cgi?pid=72461033478448&page=0&pdbId=1BMT can be shortened to http://www.rcsb.org/pdb/cgi/explore.cgi?pdbId=1BMT.

3 Copy the address of the corresponding Web page in your browser without including the accession or identification number of the current entry. Generally, this address consists of the database HTTP address and query engine, followed by a question mark (?) or equal sign (=). In the example above, you would copy the string http://www.rcsb.org/pdb/cgi/explore.cgi?pdbId=.

4 In the *Create Category* box, paste the copied information into the **External Engine** field.

Analogous to an HTTP query, where the content of a label is transmitted to a CGI script on a server, ImageMaster allows you to pass on the content of a label as the first parameter to any executable. When you double click on a label that has an executable defined in the External Engine field of the label category, the executable runs with the label content as a parameter.

To set an executable:

1 In the *Create Category* box, click on the *EXE* button located next to the **External Engine** field.

2 Browse the directory where the executable is located and select the correct file name (which should carry the extension .exe).

3 After clicking *Open*, the file, with its complete file path, automatically inserts into the **External Engine** field.

Please note that you can define a different query engine for each label category and therefore you can link one protein spot to different database entries.

Display properties
User-defined categories use a gray background color by default. You can change the default color by clicking on the color box. The new background color is used for all labels of that specific category in the active workspace.

8.3.2 Creating sets

The predefined category **Set:** was created to help you mark spots with common properties by indicating that they belong to a set. The labels in such a category do not contain specific information. They only display the name of the set to which they belong (Figure 8-1).

To create a set:

1 Select one or more spots.

2 Choose *Edit* > *Annotations* > *Add Label* from the menu.

3 In the *Add Label* box, click on the category called **Set:** and add a keyword, which will become the name of the set. For instance, to mark spots that should be exported to a spot excision robot, the final category name can be Set:Pick.

4 A label containing the name of the set (for example Pick) is attached to the selected spots (note that the string Set: is not displayed on the labels).

Normally, when a new category is created, it is immediately shown on the screen. Newly created sets are an exception to this rule. A new set is visible or hidden depending on the current visibility state of the generic category Set:.

8.3.3 Creating specific links

As seen earlier, it is possible to link labels to remote database entries by defining an external search engine for a particular category (see Section 8.3.1). In addition

to this functionality, ImageMaster offers additional possibilities to link your gel objects to Web pages, mass spectrometry data, text files or any other files located locally or on the Internet. By using specific keywords in the labels of any category, you can create links to particular objects including Web pages, files and text.

To create a specific link (for example, to a Web page, file or text), you should add an annotation to a gel and include the following items in the label field:

- A **short descriptor** that will be the visible part of the label.
- A **keyword** indicating the type of link (http:, file:, text:).
- A **link content**, which contains the information necessary to establish the link or the content of the link (in the case of a text link).

To indicate that a label is linked, its short descriptor is followed by three dots. When you double click on such a linked label, you do not enter the editing mode, as usual, but the link (Web page, file or text) is automatically opened with the appropriate software. Alternatively, to open any linked label, choose *Show* > *Annotations* > *Linked Data* in the menu.

Note that you can define links in any label category but you can only have one link per label.

Http link:
You can link spots or pixels to specific Web pages. A double click on an http-linked label will launch your default Internet browser and automatically call the corresponding Web page.

You can, for instance, create a direct link to the ExPASy Proteomics Server (Figure 8-3). In this case, the label content should contain the string "http:" followed by the address of the Web page.

File link:
You can link spots or pixels to software files. Double clicking on a file-linked label launches the specified file with the default system application associated to the file extension. The linked files can be placed in a specific directory, which is defined by choosing *Tools* > *Options* in the menu and by setting the **Annotations folder** in the *Database* tab. In this case, you only need to give the name and file extension when creating the link. Alternatively, you can link labels with files located anywhere on your system. You should then include the complete file path when referencing the file.

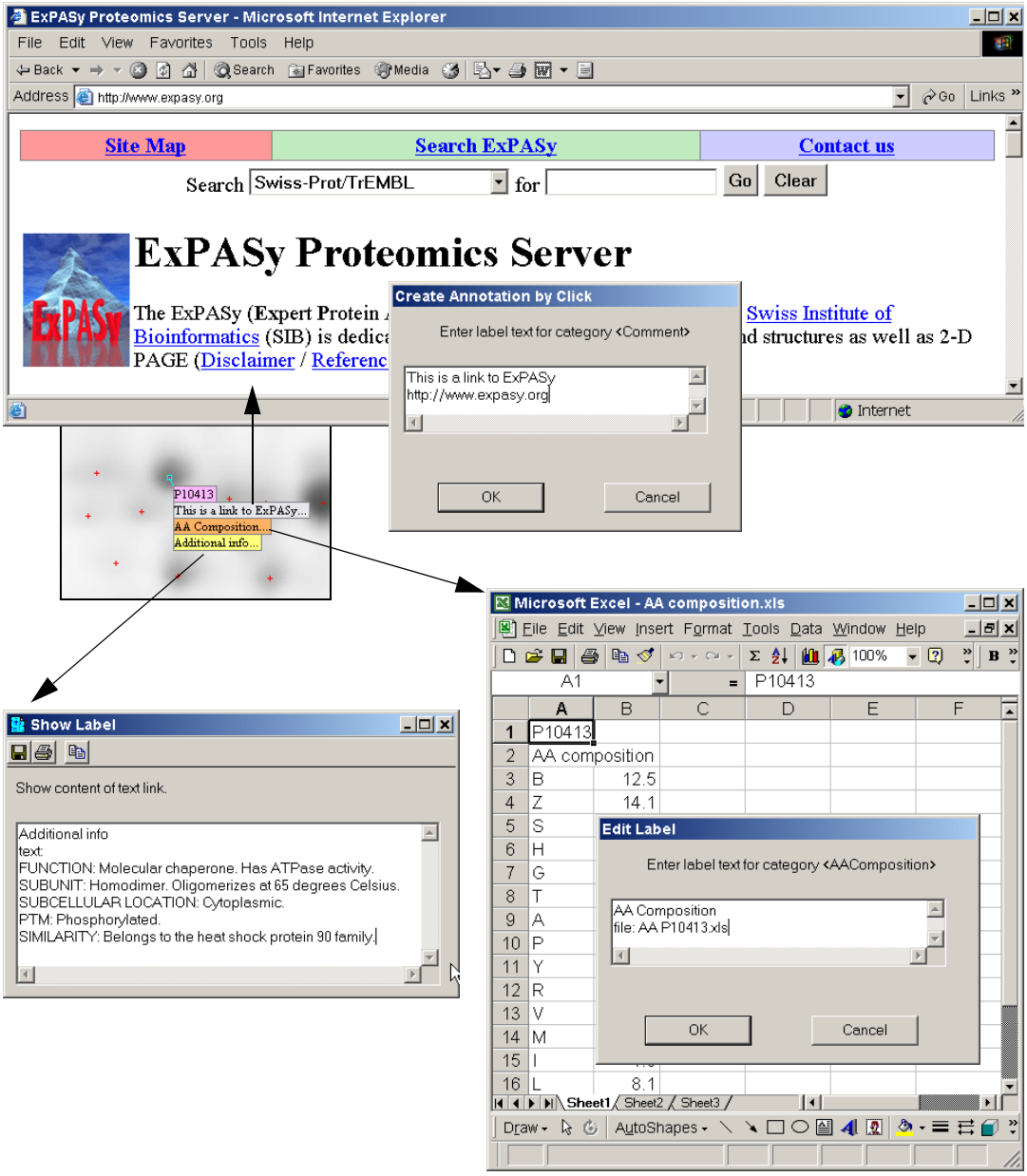


Figure 8-3. The annotation on the spot containing protein P10413 includes linked labels such as a link to a Web site (top), a link to a file (bottom right) and a text link (bottom left).

Note that you can set a different Annotation folder for each workspace. You can also create subfolders in the Annotation folder to arrange your files. The file names indicated in the labels must then contain the name of the subfolder (for example, AA composition\AA P10413.xls).

For example, you can link a protein spot to an Excel file containing the amino acid composition of a protein (Figure 8-3). The label should then consist of the string "file:" followed by the file name (with its extension).

Text link:
In some cases, you might want to associate a long text comment with a specific protein spot, but without overloading the display. The solution is to create a text link, rather than a very long annotation label. Double clicking on the linked label is sufficient to display a dialog box containing the entire text (Figure 8-3).

Text links are particularly useful to attach the bibliographic references for a spot, for instance, or any other comment such as the one in Figure 8-3. Please note that the string "text:" must first be inserted, followed by the text you would like to associate with the spot.

To connect general information about the gel, other tools are better adapted. Comments can be attached to match sets and classes, folders and projects in the workspace, as well as to any report that is saved on the hard disk. Additionally, you can specify gel descriptions (see Section 6.7.2).

8.4 Selecting annotations and labels

8.4.1 Annotation tool

Annotations and labels can be selected with the *Annotation* tool as long as they are visible on the gel. The selected labels or annotations are highlighted in green and displayed in front of the other annotations (Figure 8-4). If the annotation is attached to a spot, the spot is also selected. Remember that the opposite is also true; if you select a spot with the *Spot* tool, the attached annotation is selected as well.

To select a label, make sure the *Annotation* tool is activated and then click on the label. You can select more than one label by using the Shift key.

To select an annotation, activate the *Annotation* tool and click on the annotation basis. To select more than one annotation, select the first one, then hold down the Shift key and select additional annotations.

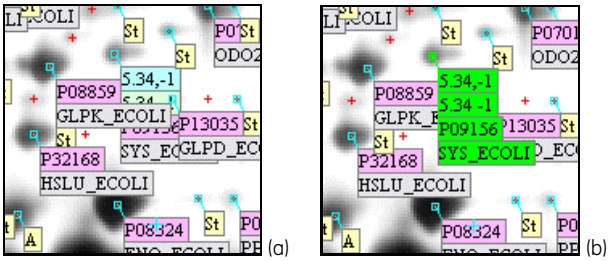


Figure 8-4. (a) Annotation hidden by some other annotations. (b) Same annotation displayed in front of all other annotations, after selection.

To select all annotations in a region, position the cursor at the top left position of the desired region, hold down the left mouse button, and then drag the cursor to the bottom right position. All annotations in the selected region are selected and highlighted in green, even those that are hidden. To select annotations in more than one region, hold down the Shift key while selecting additional regions.

To deselect all annotations, select the gels for which you would like to deactivate all annotations and click on a gel (not on an annotation).

8.4.2 Select menu

In order to select all spots and annotations in the selected gel, choose *Select* > *Select All* in the menu (or *Select* > *Unselect All* to deselect all spots and annotations). You can select specific labels and annotations with the options in the *Select* > *Annotations* menu:

- **By Content**: This feature enables the selection of labels (belonging to one or several categories that must be selected) based on their content. When the **Regular Expression** box in the window is not checked, the entered string of characters is taken literally, and the program selects all labels containing this string. By activating the Regular Expression option, regular expressions can be used in the search field (see Section 8.4.2 for details). For example, to select all labels containing the string P00, you can either type the expression .*P00.* and choose the Regular Expression field in the dialog box, or type P00 and deselect the Regular Expression field.

- **By Category**: This feature enables the selection of all labels belonging to one or several categories. Use the Shift and Ctrl keys to pick several category names at a time.

- **By Color**: When a specific color was assigned to particular annotations, the annotations can be selected by choosing the corresponding color from the menu.

- **In Region**: This option allows the selection of all annotations in the active regions, that is, in all regions that were defined with the *Region* tool on selected gels.
- **From Report File**: If any report containing information about labels or annotations (and enclosing the columns X, Y and GelName) was previously saved, this function enables the reselection of all labels contained in the selected report file. Reports on Categories, Labels, Annotations and even Spots can be used.
- **All**: This highlights all annotations in the selected gels.
- **Combine**: This option can be used to combine the current selection of annotations with a second criterion based on the Intersection, Union, Difference or Exclusion of a particular category. Please see Section 8.4.2 for more information on this feature.
- **Common Labels**: This option allows the retrieval of all sets of identical labels within a gel or among a series of gels. When two or more gels are selected, this returns the common labels for all the categories chosen in the pop-up dialog box. When only one gel is selected, this operation corresponds to selecting packed labels, that is, labels from the specified categories containing the same content (see Section 8.5.5).
- **Inverse Selection**: If some labels are already selected, this option deactivates the selected labels and selects all previously unselected ones, thus inverting the selection criteria.

Regular expressions

Regular expressions provide a mechanism to select specific strings from a set of character strings. Regular expressions use symbols and syntax elements to describe a generalized pattern. ImageMaster invokes the standard Extended Regular Expressions to search patterns in labels, the essentials of which are summarized in Table 8-1.

Syntax	Description	Example
.	Matches any one character.	**e.oli** matches:eaoli, eboli, ecoli...
[...]	Matches any character listed between the brackets. [a-z] indicates the range of characters between A and Z and [0-9] is any numeral from 0 to 9.	**P[a-d]** matches:Pa, Pb, Pc, Pd
[^...]	Matches any character except those listed between the brackets.	**P[^bd]** matches:Pa, Pc, Pe but not Pb or Pd
?	Matches the preceding element zero or one times.	**P0?1** matches:P1 and P01.
+	Matches the preceding element one or more times.	**P0+1** matches:P01, P001, P0001, ...
*****	Matches the preceding element zero or more times.	**P0*1** matches:P1, P01, P001, P0001, ...
{n}	Matches the preceding element exactly n times.	**P0{3}1** matches:P0001 but not P01 or P001
{n,}	Matches the preceding element at least n times.	**P0{2,}1** matches:P001, P0001, ... but not P01
{n,m}	Matches the preceding element at least n times, but not more than m times.	**P0{1,3}1** matches:P01 P001, and P0001, but not P1 or P00001
()	The characters between parentheses form a subexpression.	**P(24)+** matches:P24, P2424, P242424, ...
|	Matches the expression before or after the vertical line. Mostly used within a subexpression.	**P(ab|cd)1** matches:Pab1 and Pcd1
^	A circumflex outside a bracket expression anchors the element it starts with to the beginning of a string; such an element can match only a sequence starting at the first character of a string.	**^(ec).*** matches:ecoli, ecoli_eftu but not eftu_ecoli

Syntax	Description	Example
$	A dollar sign outside a bracket expression anchors the element it terminates with to the end of a string; such an element can match only a sequence ending at the last character of a string.	**.*(ecoli)$** matches:ecoli, eftu_ecoli but not ecoli_eftu

Table 8-1. Regular expressions available to search patterns in labels. Please not that the term element used in the description indicates a character or a subexpression.

The characters **^.[$()|*+?{** have a special meaning in certain contexts. You must exclude them from the expression with a backslash (****) if you want them to be taken literally. This means that if your labels contain any of these special characters, you must precede them with a backslash if you want to include them as normal characters in your search expression. Note that you must also release the backslash character itself from the expression. For example, the search pattern **R*.*** returns the result **R*3.24** but not **R/2.87**.

Nevertheless, bracketed expressions are an exception to the rule. Inside bracketed expressions, all special characters, including the backslash, lose their special meaning (for instance, **[*\+?{}.]** matches exactly any of the characters inside the brackets).

The order of precedence for the regular expressions described above is as shown in Table 8-2 . For example, the regular expression **abc2|3de** matches either the string **abc2** or the string **3de** (rather than the string **abc2de** or **abc3de**) because concatenation has a higher order of precedence than alternation.

Escaped characters	\<Special character>
Bracket expression	[ ]
Grouping	( )
Single-character duplication	* + ? {m,n}
Concatenation	
Anchoring	^ $
Alternation	|

Table 8-2. Order of precedence (from high to low) for regular expressions.

Combining sets of annotations

You can select annotations based on multiple criteria. You can do this by combining sets of annotations in particular ways. You can select a first set of

annotations on your gels and then combine this set with a second set according to one the following schemes (see also Figure 8-5):

- **Intersection**: Selects spots that are in the intersection of two criteria.
- **Union**: Selects spots that belong to either one criterion or the other.
- **Difference**: Selects spots that belong to either of the two criteria, but excludes those that belong to both of them.
- **Exclusion**: Selects spots that are found only in the first criterion and are not found in the second criterion.

The possibility of combining sets of annotations is particularly useful when you have different sets defined. Imagine, for example, that you marked interesting spots found from Factor Analysis with labels of the category **Set:Factor** Analysis. Similarly, you marked significant spots emerging from a Student t test with labels of the category **Set:Student t test**. You could now select all spots from the first set by choosing *Select* > *Annotations* > *By Category* from the menu, and picking **Set:Factor Analysis** from the *Select Labels by Category* box. When you subsequently choose *Select* > *Annotations* > *Combine* > *Intersection* from the menu, and pick the category **Set:Student t test**, only spots that belong to both sets remain selected.

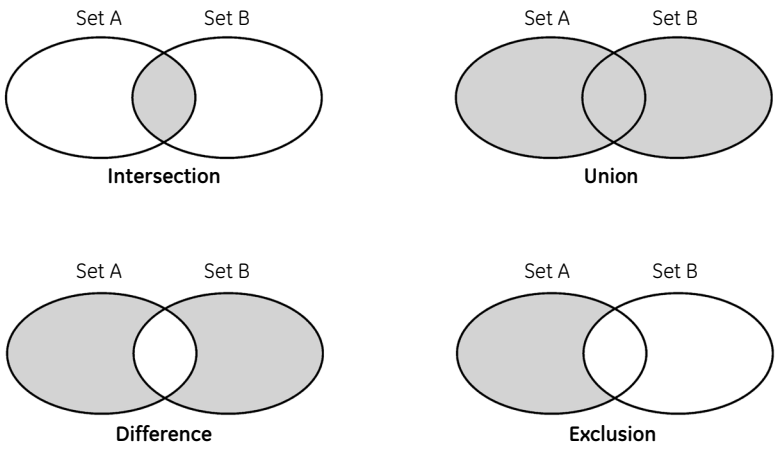


Figure 8-5. Combining sets of spots in ImageMaster.

To combine sets of annotations:

1 Select annotations according to a first criterion by using the options in the menu or by picking the desired annotations with the *Annotation* tool.

2 Choose *Select* > *Annotations* > *Combine* in the menu, and select one of the four possible operations (**Intersection**, **Union, Difference** or **Exclusion**).

3 Choose the label category corresponding to your second criterion in the dialog box, and click *OK*.

4 The result of the logical operation is selected according to Figure 8-5.

8.4.3 Reports

You can select labels or annotations directly from a displayed report on Annotations, Labels, Categories or even Spots (at least for spots that are linked to annotations). Do this by double clicking on an item in the report or by selecting one or several items and clicking the *Select on Gels* icon in the report toolbar.

8.5 Displaying annotations and labels

You can change the way annotations and labels are displayed but these modifications will not be saved in your ImageMaster file.

8.5.1 Annotation flag position

Sometimes you may want to move an annotation flag because you are preparing an illustration or just want to see what lies underneath. To interactively change an annotation's flag position, click on one of the labels, and drag the flag to the desired position (Figure 8-6).

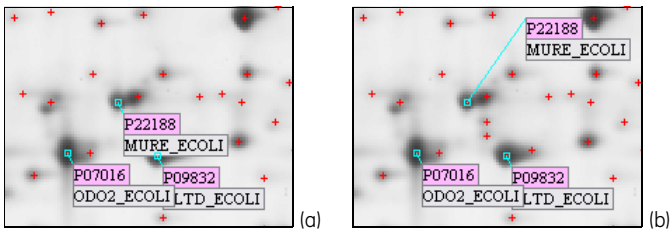


Figure 8-6. (a) Default and (b) modified annotation flag position.

8.5.2 Annotation flag color

As mentioned inSection 8.2 each predefined label category is automatically created using a different background color (pink for the Ac category, blue for landmarks, etc.), to help you visually identify the various label categories. You can change the default colors by going to the *Categories* tab in the ImageMaster *Options* (accessible by choosing *Tools* > *Options* in the menu), selecting an annotation category, and modifying its **Color** in the Display properties. The new

background color is used for all labels of that specific category in the current workspace.

8.5.3 Annotation flagpole color

Annotation flagpoles can be displayed on the screen using three different colors other than the default cyan.

To change the color of the annotation flagpoles:

1 Select the annotations for which you would like to change the flagpole color.

2 Choose *Show* > *Annotations* > *Set Color* in the menu, and select one of the proposed colors. Please note that the *Default* option corresponds to cyan.

3 The flagpoles of the selected annotations are displayed with the chosen color (after deselecting the spots).

4 The colors remain displayed this way until you choose *Show* > *Annotations* > *Set Color* > *Reset All* to revert all selected annotations to the default cyan, or until you exit ImageMaster.

8.5.4 Show / hide annotations and labels

You can quickly cover entire gel images with a considerable number of annotations and labels, which are not always necessary at any given moment in the analysis. Therefore, you can hide all or part of them. ImageMaster not only gives you the possibility to hide certain label categories; the program also allows you to hide certain selected annotations (Figure 8-7).

Note that the visibility of an annotation or label plays a role in its selection. As a rule, annotations and labels can only be selected and manipulated with the *Annotation* tool when they are visible on the gels. To select hidden annotations or labels, the menu options should be used.

To show or hide all annotations and spots in the selected gels, choose *Show* > *Show All* (or *Show* > *Hide All*) in the menu. Hide All also hides the match vectors.

To show or hide specific annotations, you can use one of the following options available in the *Show* > *Annotations* menu.

- **Show All**: Makes all the annotations on the selected gels visible.
- **Hide All**: Hides all the annotations on the selected gels.

- **In Region**: Shows all annotations in the area selected with the *Region* tool. This option is handy when all annotations were previously hidden.
- **For Spots**: Shows all the annotations linked to selected spots. This feature is particularly useful when all annotations were previously hidden.
- **Only Selected**: Shows only the selected annotations and hides all the other ones.
- **Hide Selected**: Hides the selected annotations.

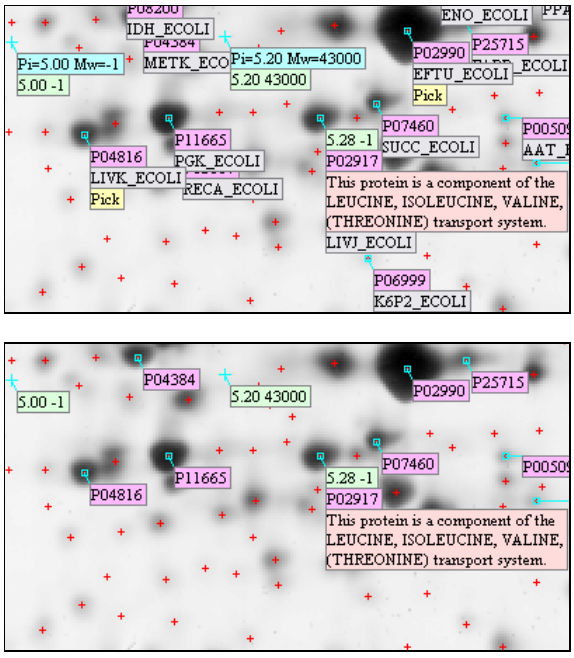


Figure 8-7. (a) All categories and labels are visible. (b) Only the Ac, pI_MW and Comment categories are visible, and only for particular spots.

To show or hide specific categories:

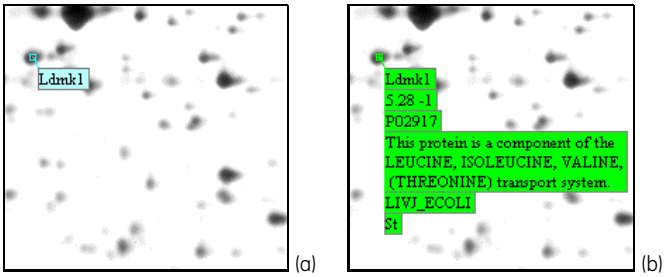
1 Select the gels for which you would like to change the category visibilities.

2 Choose *Show* > *Annotations* > *Visible Categories* from the menu.

3 In the *Set Categories Visibility* box, the check boxes of the visible categories are selected; those of hidden categories are empty, whereas grayed check boxes correspond to categories that take different visibility states in the various gels (hidden in some gels, shown in others).

4 To hide a category, make sure its box is unchecked. On the other hand, select an empty check box to show the corresponding category. You can click once or twice in a grayed check box to hide or show the corresponding label category in the selected gels.

When you click on an annotation that has both visible and hidden labels, all of its labels are displayed on the screen during the time it remains selected (Figure 8-8). The hidden labels immediately disappear when the annotation is deselected.

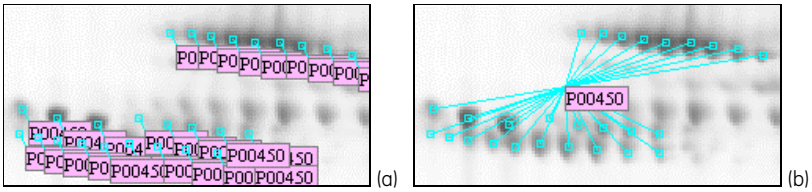


(a) (b)

Figure 8-8. (a) Unselected annotation with hidden labels. (b) When the annotation is selected the hidden labels become visible.

8.5.5 Pack / unpack categories

By default, each spot is displayed with its own label, even if several labels hold the same content. However, to declutter the display, you can show a single label for all identical labels. In ImageMaster, this option is called packing categories (Figure 8-9).



(a) (b)

Figure 8-9. (a) Unpacked and (b) packed labels for protein P00450 in the Plasma Master Gel (SWISS-2DPAGE database). The labels belong to the predefined Ac category.

To pack categories:

1 Select the gels for which you would like to pack one or more categories.

2 Choose *Show* > *Annotations* > *Packed Categories* in the menu.

3 In the *Set Packed Categories* box, the check boxes of the packed categories are selected; those of unpacked categories are empty,

whereas grayed check boxes correspond to categories that have different packing states in the various gels.

4 To pack a category, check its box. Deselect the check box to unpack a category. Finally, you may need to click once or twice in a grayed check box to unpack or pack the corresponding category.

8.6 Adding / modifying annotations and labels

You can add and modify annotations and labels in different ways using the *Annotation* tool, *Edit* menu options, *Reports* or *File > Import* function. The *Annotation* tool is helpful if you want to add or modify a single label or just a few labels. The *Edit* menu options are more adapted to the creation of a large number of annotations or labels simultaneously. Reports are useful for editing existing annotations.

8.6.1 Annotation tool

When double clicking on a label while the *Annotation* tool is activated, the *Edit Label* box is displayed. Change the text in this box to modify your label content.

The *Annotation* tool should also be used to change the position of an annotation. In this case, simply select an annotation and drag its basis to the new location. When you drag an annotation linked to a spot, outside of that spot, ImageMaster asks if you want to remove the link with the spot. Similarly, when you move an annotation onto a spot, ImageMaster asks if you want to link it with that spot.

8.6.2 Edit menu

Various options for modifying, copying, pasting, duplicating and deleting labels or annotations are available from the *Edit > Annotations* menu. With the exception of the *Modify* function, all of these features can be used to edit (add, delete, modify) several annotations at a time.

Modify

Once an annotation is selected, you can change the content of its labels and add new categories by choosing the *Modify* option and entering the desired modifications in the *Edit Label* box. Please note that only one annotation can be selected at a time. When picking a single label, only the content of the selected label can be modified.

Copy / paste annotations

You can select annotations in a given gel image and copy them to the corresponding locations in other gels. This is a simple means of creating a set of similar annotations in a series of gels, and can be useful for quickly adding landmarks, for instance. Subsequently, you just need to adjust the annotation positions.

To copy annotations from one gel to a set of other gels:

1 Select the annotations you would like to copy to other gels.

2 Choose *Edit* > *Annotations* > *Copy* in the menu. The selected annotations are copied into memory.

3 Select the gels into which you want to copy the annotations.

4 Choose *Edit* > *Annotations* > *Paste Annotations* in the menu.

5 The copied annotations are pasted to the corresponding locations in the selected gels. These locations are estimated by interpolation between the two nearest common landmarks (landmarks with the same name). When no such landmarks exist, the annotations are placed at the same image location (X and Y coordinates).

6 Adjust the annotation positions using the *Annotation* tool.

Copy / paste labels

Instead of copying entire annotations, you can also copy distinct labels from one gel to selected spots or annotations in a series of gels.

To copy labels from one gel to a set of other gels:

1 Select the labels you would like to copy.

2 Choose *Edit* > *Annotations* > *Copy* in the menu. The selected labels are copied into memory.

3 Select the spots or existing annotations into which you want to copy the labels.

4 Choose *Edit* > *Annotations* > *Paste Labels* in the menu.

5 The copied labels are pasted to the selected spots and annotations.

Copy matched labels

When gels have been matched, labels existing in one gel can be copied to their corresponding spots in other gels. This is particularly useful when you have annotated your master gel (for instance, by adding and modifying annotations to the master gel via an Intra-class Report) and want to propagate the labels to all matched gels.

To copy matched labels from one gel to a set of other gels:

1 Select the labels to be copied in the source gel.

2 Select the source gel and the gels into which you want to copy the labels.

3 Choose *Edit* > *Annotations* > *Copy Matched Labels* from the menu.

4 The selected labels are copied to the corresponding spots in the other gels.

Duplicate labels

You can copy selected labels to another category. Since the selected labels may belong to different categories, this option can be used to merge several categories into a new one. However, only one label per annotation can be duplicated at a time.

To duplicate labels:

1 Select the labels you would like to duplicate (as part of another category).

2 Choose *Edit* > *Annotations* > *Duplicate Labels* from the menu.

3 In the *Duplicate Labels* box, pick the recipient category, or enter a new category name.

4 The selected labels are copied to the chosen category.

5 When a label for the chosen category already exists, ImageMaster asks whether you want to keep it or replace it with the new one.

Rename category

ImageMaster allows you to rename any category, be it a predefined or user-defined category, or a Set:. In the case of a Set:, the label contents (name of the Set:) are automatically updated on the gel.

To rename a category:

1 Select the gels for which you would like to rename a category.

2 Choose *Edit* > *Annotations* > *Rename Category* in the menu.

3 In the displayed category list, select the one you would like to rename.

4 Enter the new category name in the next dialog box.

5 ImageMaster immediately applies the modifications to all labels on the selected gels.

Deleting annotations and labels
Two possibilities are available from the *Edit* > *Annotations* menu for deleting selected labels or annotations:

- **Delete by Category**: With this feature, only the selected labels belonging to the categories specified from the category list are deleted.
- **Delete**: This option deletes all selected labels. Please note that if you select annotations, all their labels are selected and therefore deleted.

8.6.3 Reports

This section describes the functionalities in ImageMaster that are helpful for editing annotation and label content in reports that contain information on these objects (see Section 8.7).

Please see Chapter 5 for details about other standard tools available in the various report toolbars, including icons to save, print or copy reports or report lines, to navigate between reports and gels, to refine report selections, and so on.

Creating annotation categories
Annotation Reports, Intra-class and Inter-class Reports contain the *Annotate* icon in their toolbar. Clicking on the *Annotate* icon allows you to insert a category as an extra column, by selecting its name from the category list (containing all the categories present in the open gels). You can create a new category by typing its name in the upper field of the displayed window. In this case, you are asked for the category constraints.

Adding or modifying labels
Once you created or displayed the desired categories, you can directly add new labels to the appropriate cells, or edit existing ones. Just double click in a cell to start typing your label. When finished, a single click in any cell quits the editing mode. The cell corresponding to the modified label appears in dark green when the related line is selected, or in gray otherwise. You can quickly see that modifications were made to your report when an asterisk follows its window title.

Please note that when you want to maintain a current selection while editing labels, you should hold down the Shift key while double clicking in a cell. You can release the Shift key as soon as you are in the editing mode.

Notice that categories of the type Set: or categories using the Boolean Data Type are displayed in the form of check boxes in some reports. Editing their labels corresponds to checking the box, in order to indicate whether the corresponding item belongs to a Set: (checked box) or not (empty check box), or takes the value 0 (empty check box) or 1 (checked box) for a Boolean Data Type.

To check several boxes (rows) simultaneously within a given category (column):

1 Select the rows for which you would like to change the checked state.

2 Hold down the Shift key while clicking in the check box of one of the selected rows (obviously in the relevant column). All selected lines are now in the same checked state. Click in the check box a second time if the state should be inversed.

Updating gels
Subsequently, the *Update Gel* icon should be used to make the modifications definitive and to propagate the information to your gels. Note that the cells of updated labels return to their original color. When all labels in your report are updated, the asterisk behind the report title also disappears.

NOTE! You should be aware of the fact that the annotations displayed in the Intra-class and Inter-class Reports are those from the master gel. This means that any modifications, including the creation of new columns corresponding to categories and the addition of new labels, can only be applied to the master gel. Note that this is not really a problem, since you can easily propagate selected labels to matched spots (see Section 8.6.2).

8.6.4 Importing labels and annotations

You can import annotations and labels from a file into open gels. You can import labels from a *Label Report*, for example, or from any other tab-delimited text file containing the columns Category, Label, SpotID, X and Y. If the Spot ID is not known, use -1 in this field and ImageMaster will position the label in the corresponding X and Y positions of the gel. If X and Y positions are not known, use -1 in these fields and ImageMaster will position the label on the spot with the corresponding Spot ID.

To import labels into open gels:

1 Select the gels for which you would like to import labels.

2 Choose *File* > *Import* > *Labels* in the menu.

3 Browse the folder containing the desired Label Report (.rpt) or a text file (.txt) containing the label information. Click *OK*.

4 ImageMaster asks for confirmation. Answer *Yes* if you want to proceed.

5 The imported labels are added to the selected gels.

Similarly, you can import annotations from an *Annotation Report* or a tab-delimited text file containing the required columns: SpotID, X, Y, and a column for each category to be imported.

To import annotations into open gels:

1 Select the gels for which you would like to import annotations.

2 Choose *File* > *Import* > *Annotations* in the menu.

3 Browse the folder containing the desired Annotation Report (.rpt) or a text file (.txt) containing annotation information. Click *OK*.

4 ImageMaster asks for confirmation. Answer *Yes* if you want to proceed.

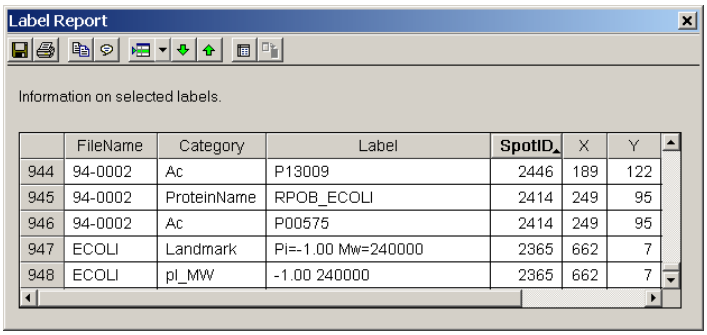
5 The imported annotations are added to the selected gels.

8.7 Annotations and labels in reports

ImageMaster provides various reports including specific information about annotations, labels, and categories. Intra-Class and Inter-Class Reports can display the content of annotations and labels attached to spots in the master gel. As pointed out in Chapter 5, these reports are editable, although to different extents. In Section 8.6.3 you will find that the editing feature is particularly useful for adding or modifying annotations and labels.

8.7.1 Label report

The *Label Report* displays information about selected labels, such as the gel name, category, label content, Spot ID, and X and Y coordinates (Figure 8-10). The Label column in this report is editable, thus giving you the possibility to modify the existing label contents.

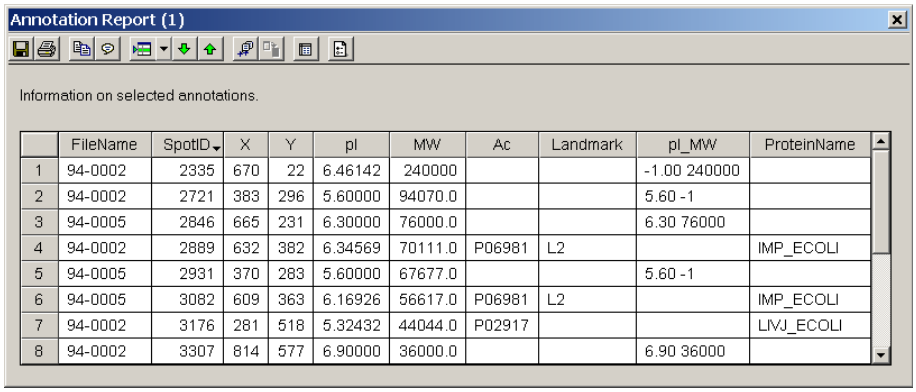


	FileName	Category	Label	SpotID	X	Y
944	94-0002	Ac	P13009	2446	189	122
945	94-0002	ProteinName	RPOB_ECOLI	2414	249	95
946	94-0002	Ac	P00575	2414	249	95
947	ECOLI	Landmark	Pi=-1.00 Mw=240000	2365	662	7
948	ECOLI	pI_MW	-1.00 240000	2365	662	7

Figure 8-10. Report on selected labels in selected gels.

8.7.2 Annotation report

The *Annotation Report* displays information on selected annotations in selected gels. Thus, for each annotation, you will find the gel name, Spot ID, X and Y coordinates, and the label contents for all chosen categories (Figure 8-11). You can modify the label contents for each category, or even add new categories.

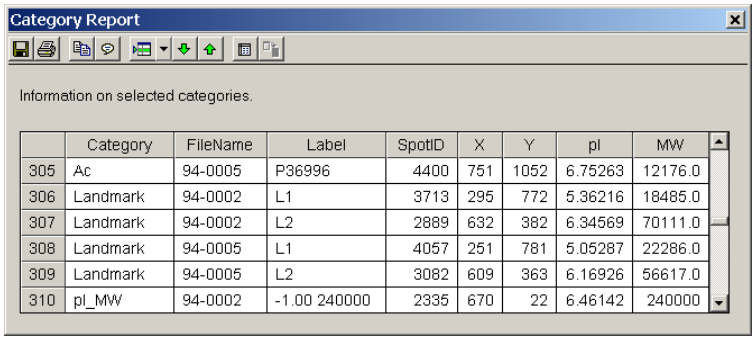


	FileName	SpotID	X	Y	pI	MW	Ac	Landmark	pI_MW	ProteinName
1	94-0002	2335	670	22	6.46142	240000			-1.00 240000	
2	94-0002	2721	383	296	5.60000	94070.0			5.60 -1	
3	94-0005	2846	665	231	6.30000	76000.0			6.30 76000	
4	94-0002	2889	632	382	6.34569	70111.0	P06981	L2		IMP_ECOLI
5	94-0005	2931	370	283	5.60000	67677.0			5.60 -1	
6	94-0005	3082	609	363	6.16926	56617.0	P06981	L2		IMP_ECOLI
7	94-0002	3176	281	518	5.32432	44044.0	P02917			LIVJ_ECOLI
8	94-0002	3307	814	577	6.90000	36000.0			6.90 36000	

Figure 8-11. Report on selected annotations in selected gels.

8.7.3 Category report

You can display information about all labels belonging to selected categories and selected gels. The information displayed in this report (Figure 8-12) is the same as for a report on labels (Figure 8-10) and the label contents can be edited as well.



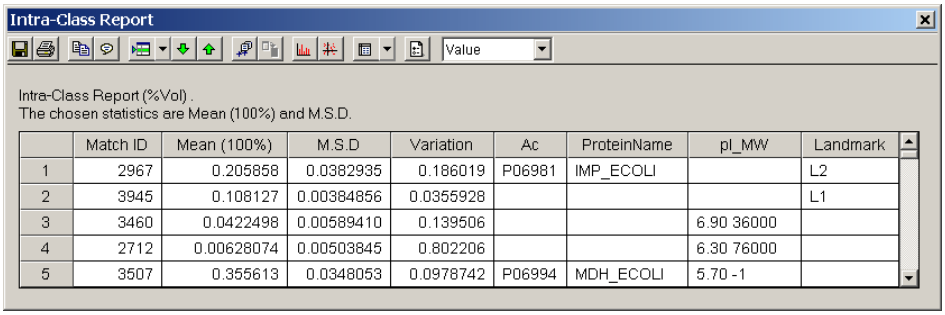
	Category	FileName	Label	SpotID	X	Y	pI	MW
305	Ac	94-0005	P36996	4400	751	1052	6.75263	12176.0
306	Landmark	94-0002	L1	3713	295	772	5.36216	18485.0
307	Landmark	94-0002	L2	2889	632	382	6.34569	70111.0
308	Landmark	94-0005	L1	4057	251	781	5.05287	22286.0
309	Landmark	94-0005	L2	3082	609	363	6.16926	56617.0
310	pI_MW	94-0002	-1.00 240000	2335	670	22	6.46142	240000

Figure 8-12. Report on selected categories in selected gels.

8.7.4 Intra-Class Report

An *Intra-Class Report* contains information about selected matches and their comprised spots (Match ID, central tendency, dispersion, spot values, coefficient

of variation, range ratio, separability, etc.). In addition, they can be used to display the labels attached to the spots in the master gel. In an Intra-Class Report, you can add labels or edit existing ones and create new columns corresponding to categories (Figure 8-13).

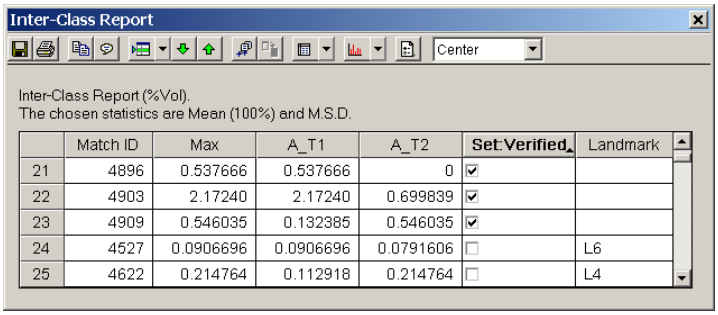


	Match ID	Mean (100%)	M.S.D	Variation	Ac	ProteinName	pI_MW	Landmark
1	2967	0.205858	0.0382935	0.186019	P06981	IMP_ECOLI		L2
2	3945	0.108127	0.00384856	0.0355928				L1
3	3460	0.0422498	0.00589410	0.139506			6.90 36000	
4	2712	0.00628074	0.00503845	0.802206			6.30 76000	
5	3507	0.355613	0.0348053	0.0978742	P06994	MDH_ECOLI	5.70 -1	

Figure 8-13. Report on selected matches in selected gels.

8.7.5 Inter-Class Report

An *Inter-Class Report* provides statistical values for sets of gels, calculated for all selected matches, such as central tendency, dispersion and overlapping measures. In such a report, you can display the labels attached to the spots in the master gel, add labels or edit existing ones and create new columns corresponding to categories (Figure 8-14).



	Match ID	Max	A_T1	A_T2	Set:Verified	Landmark
21	4896	0.537666	0.537666	0	☑	
22	4903	2.17240	2.17240	0.699839	☑	
23	4909	0.546035	0.132385	0.546035	☑	
24	4527	0.0906696	0.0906696	0.0791606	☐	L6
25	4622	0.214764	0.112918	0.214764	☐	L4

Figure 8-14. Report on selected matches in selected classes.

9 Matches

9.1 Introduction

Matching is a key operation in 2-DE image analysis. Basically, the image matching algorithm compares gel images to find Matches between related spots, that is, spots representing the same protein in the gels. A match is therefore composed of spot n-tuples (S1, S2, ..., Sn) where S1 is a Spot ID in the first gel, ..., and Sn a Spot ID in the last gel.

To be able to initiate the matching process in ImageMaster 2D Platinum 6.0, gels must be part of a Match Set. A match set includes gels or populations of gels (in sub match sets) that should be compared and therefore matched together.

Every match set is represented by a Master. This master image is created by ImageMaster based on the Reference for the match set, chosen by the user. Essentially, the Master is a copy of the match set reference and contains the same spots, which are therefore automatically matched between the two images. But once other gels in the match set are matched with the Master, it is possible to copy additional spots from the matched gels to the Master.

As briefly described above, a match set can include gels, but also populations of gels represented by a sub match set. The principle can be illustrated with Figure 9-1. The gels A1, A2 and A3 are part of Match Set A. This match set is represented by the master image Master A, based on the selected reference image A1 (in red). Since Master A is a copy of A1, it contains the same spots. The same is true for Master B, which represents match set B.

Once all the gels in match set A are properly matched with Master A, and the gels in match set B are matched with Master B, the match sets A and B can be compared. To do so, they are included in a higher level match set AB, for which match set A was chosen as a reference. In practice, comparing match sets A and B amounts to comparing their master gels, Master A and Master B. The new representative Master of this match set, Master AB, is an automatically created copy of the master image from the selected reference match set.

The interesting thing about this procedure is that matches are propagated at each level. This means that once all match sets (in this case, A, B and AB) are effectively matched, spots from gel A2, for instance, can be directly compared with those from gel B3, through the existence of a common node (Master AB) in the matching tree.

NOTE! The master images are the nodes for the propagation of matches. If a protein is not present in the Reference, and so on the Master, no matches will be formed, even if the protein appears on all other gels (see blue spots in Figure 9-1). Consequently, the protein will not turn up in the analysis. In the example of Figure 9-1, choosing one of the other gels (A2 or A3) as the Reference would have been more appropriate. To solve this problem, spots from matched gels can be copied to the master gel.

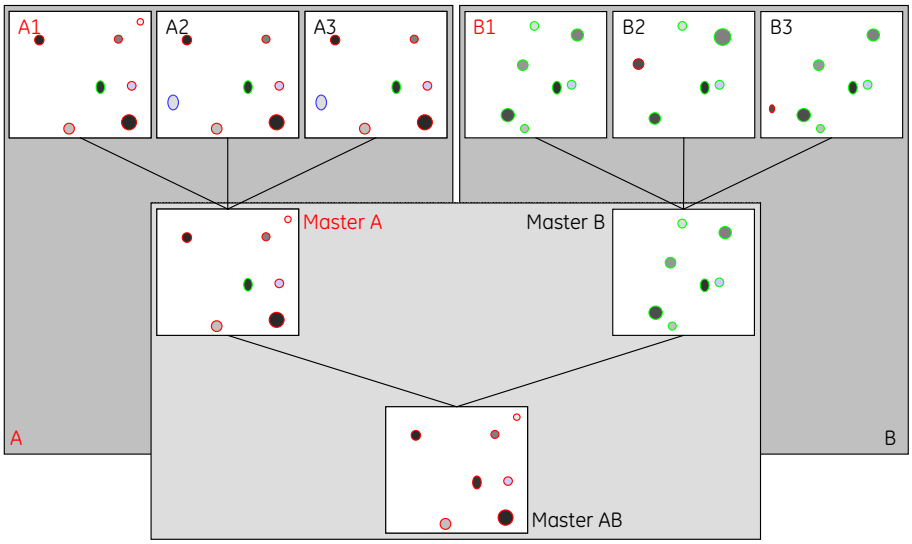


Figure 9-1. Population matching and the importance of the master image. Matches are selected by choosing the corresponding spot, which will appear in green. One match is selected in match set A. In match set B, all matches are selected. Representative spots in a population that are not in the reference gel (blue spots) should be manually copied to the Master.

When several gels are matched to a given Master, this Master provides a unique numbering scheme for spots across all gels. Each spot in a gel image can in fact be associated to the corresponding Spot ID in the master gel. The Spot ID in the master gel is then called the Match ID.

The fact that you are no longer limited to matching gels, but now can match populations of gels is a major innovation over previous versions of the software. What is more, you can match different levels of populations. This is called population matching. The countless possibilities that population matching offers are further documented in Chapter 4. The current chapter will concentrate on the matching procedure and the manipulation of matches.

9.2 Matching

The prerequisites for matching gels or match sets is that spots are detected, the gels or sub match sets to be matched are added to a match set (see Chapter 4), and this match set is opened in a worksheet.

NOTE! A DIGE gel is an inherent match set for which the co-run images are automatically matched (see Chapter 4 to learn how to quickly create match sets for DIGE gels). To subsequently match different DIGE gels, proceed as with any other match sets.

NOTE! Instead of the reference image, the Master is displayed in a [MatchSet] worksheet. As the master and reference images are the same, and matches are automatically created between the two, the reference image is not displayed by default. To show the Reference in the match set, choose *Show* > *Show Reference*.

NOTE! The functionalities that are specifically related to editing matches and reporting on matches are only available when working in a [MatchSet] worksheet.

9.2.1 Automatic matching

ImageMaster is designed to match gels with minimum user intervention. Nevertheless, sometimes you may need to help the matching process by specifying a few starting matches or landmark annotations (see below).

Before adding any landmarks or starting matches, we recommend trying to match your gels with only a single landmark or possibly none at all. In some cases, no landmarks will be required. More often, a single landmark is sufficient for quick and efficient matching. If matching takes a long time and no matches seem to be found (the counter shown during matching always shows 0 or increases slowly), you may want to cancel the matching procedure and define a few landmarks. Subsequently, you can repeat the automatic matching procedure below.

To match the gels in a match set:

1 Open the match set in a new [MatchSet] worksheet.

2 Select the gels to be matched in the worksheet.

3 Choose *Edit* > *Matches* > *Match Gels* from the menu.

4 If matches already exist, you are asked whether you want to keep them or not. If you answer *Yes*, ImageMaster keeps the existing matches and

just looks for additional ones. If you click *No*, the matching procedure is started from scratch (only annotations of the landmark category, if any, are considered).

5 All selected gels are matched with the master image. Please note that the matching step might take some time, depending on your computer capacity.

6 When matching is completed, ImageMaster provides the total number of matches found.

NOTE! Matches are saved on disk when saving the gel files. However, the information in the workspace project is necessary to reconstruct the match sets and retrieve the matches. This is the reason why it is important to regularly save the workspace (which in turn saves the projects) and why the workspace is saved by default when closing the software.

9.2.2 Specifying starting matches

When the gels are very distorted or different, automatic matching may fail. In this case, it can be necessary to define starting matches or landmarks, that is, corresponding spots that are present in each of the gels to be matched. However, the number of landmarks or starting matches should be kept to a minimum. Often a single landmark may suffice to successfully initialize the matching procedure.

NOTE! The wise choice of landmarks or matches is imperative for obtaining adequate matching results. Landmarks essentially correct global deformations of gels. Therefore, it is recommended not to put landmarks on spots in locally distorted regions because this can worsen the matching results around such regions. To correctly match gels, you should perform a first matching round using some carefully selected landmarks. If some locally deformed regions are not properly matched, it is best to delete any wrong matches that were generated, manually add a few new matches, and then to carry out a second automatic matching cycle while keeping the existing matches.

Input matches

When you manually define starting matches (see Section 9.5.1), the matching algorithm uses these matches as matching seeds. These seeds are not permanent, however. No difference is made between user-defined matches and those found by the matching algorithm. If you restart the matching procedure without taking into account the previously found matches, the manually input matches are also not considered.

Landmark annotations
If two or more gels have annotations with identical labels, for example landmark annotations, you can use the landmarks to initiate the matching process on the condition that the annotations are linked to spots. The matching algorithm superimposes those annotations that bear the same labels (in the master gel and the matched gel) and uses them as matching seeds. Please see Chapter 8 to find out how to create annotations.

The following rules should be considered when defining landmark annotations:

- Keep the number of landmarks to a minimum.
- Only define landmarks on clearly corresponding spots. Protein variants should definitely not be used as landmarks.
- Landmarks should be placed on small, sharp spots, rather than on large, diffuse ones (to reduce the error in the position).

When the annotations are of the landmark category, ImageMaster automatically takes them into account for the matching procedure. In this case, you just have to choose *Edit* > *Matches* > *Match Gels* from the menu.

When the annotations belong to any other category, you must first match the annotations to generate starting matches. Once this is done, you can run the automatic matching algorithm again by choosing *Edit* > *Matches* > *Match Gels* from the menu. You must keep the existing pairs when asked for it.

To match annotations:

1 Select the gels for which annotations should be matched.

2 Choose *Edit* > *Matches* > *Match Annotations* from the menu.

3 In the pop-up dialog box, select the desired category to be matched.

4 ImageMaster creates matches between identical labels of the chosen category.

5 You can now run the automatic matching procedure.

9.3 Selecting matches

9.3.1 Menu options

Selecting matches consists of selecting their corresponding spots. You can select particular matches by using the items in the *Select* > *Matches* menu:

- **By ID**: Allows the selection of matches based on their Match ID.
- **For spots**: Selects all spots matched with selected spots in the selected gels.
- **All**: Selects all spots matched with spots in the master image.
- **Refine Selection**: Refines an initial match selection based on the presence or absence of matched spots in the selected gels (see below for more details).
- **Inverse Selection**: If some matches are already selected, this option deselects the selected matches and selects all the unselected ones, thus inverting the selection criteria.
- **Multiple Matches**: Selects only the multiple matches in the selected gels (see below).

Refining a match selection

ImageMaster allows you to refine an initial match selection based on the presence or absence of matched spots in the selected gels. By using the sliders in the *Refine Match Selection* window (Figure 9-2), you can choose the threshold (X) for the refinement of the matches corresponding to selected spots:

- **Selected spots is >= X**: Keeps matches that have selected spots in at least X of the chosen gels.
- **Selected spots is < X**: Keeps matches that have selected spots in less than X of the chosen gels.
- **Unselected spots is >= X**: Keeps matches that have unselected spots in at least X of the chosen gels.
- **Unselected spots is < X**: Keeps matches that have unselected spots in less than X of the chosen gels.

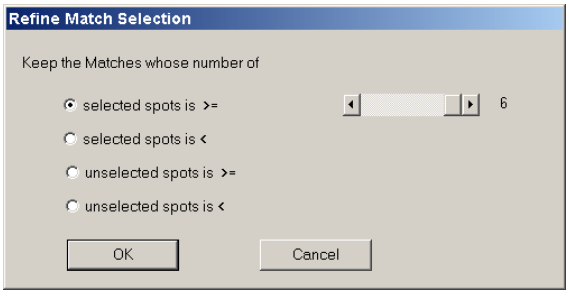


Figure 9-2. Refine a match selection based on the number of selected spots in each match.

For example, suppose you have a set of 6 matched gels, for which you would like to study the matches that contain at least 3 spots with a relative volume higher than 0.02. In other words, you would like to analyze spots according to two criteria. First, the spots should have a relative volume higher than a certain threshold, in order to select spots that are meaningful in terms of volume. Second, these spots should be present in at least half of the selected gels. You may select this special set of spots by performing the following steps:

To filter spots in matched gels:

1 Select your six matched gels. Please note that the master gel will not be included in the refinement. The reference image must be made visible to be included in the selection (choose *Show* > *Show Reference*).

2 Select all spots in the gels by choosing *Select* > *Spots* > *All* from the menu.

3 Subsequently, refine the current spot selection based on the relative spot volume, by choosing *Select* > *Spots* > *Refine by Value* from the menu, picking the **%Vol**, and keeping only the spots whose **%Vol is >= 0.02**.

4 Spots corresponding to this first criterion remain selected.

5 Choose *Select* > *Matches* > *Refine Selection* from the menu, and **Keep the Matches whose Number of Selected Spots is >=** 3 (by clicking the first option and moving the slider to the value 3).

6 The remaining matches meet both specified criteria.

As you may notice, there are endless possibilities for combining selections of spots and matches. Your own experiments and needs will drive the usage of this function.

Multiple matches

ImageMaster enables the creation of multiple matches (see Section 9.5 for details about adding matches). In contrast to a single match, where one spot in a master gel is matched with exactly one spot in the other gels, a multiple match implies that a single spot in the master gel can be matched to several spots in another gel (Figure 9-3). The opposite case (several spots in the Master matched with a single spot in another gel) is not possible.

The spots from such multiple matches (on a given gel image) are considered as a single spot in the subsequent data analysis. The calculated quantification values reflect the size, intensity and abundance of the combined spots.

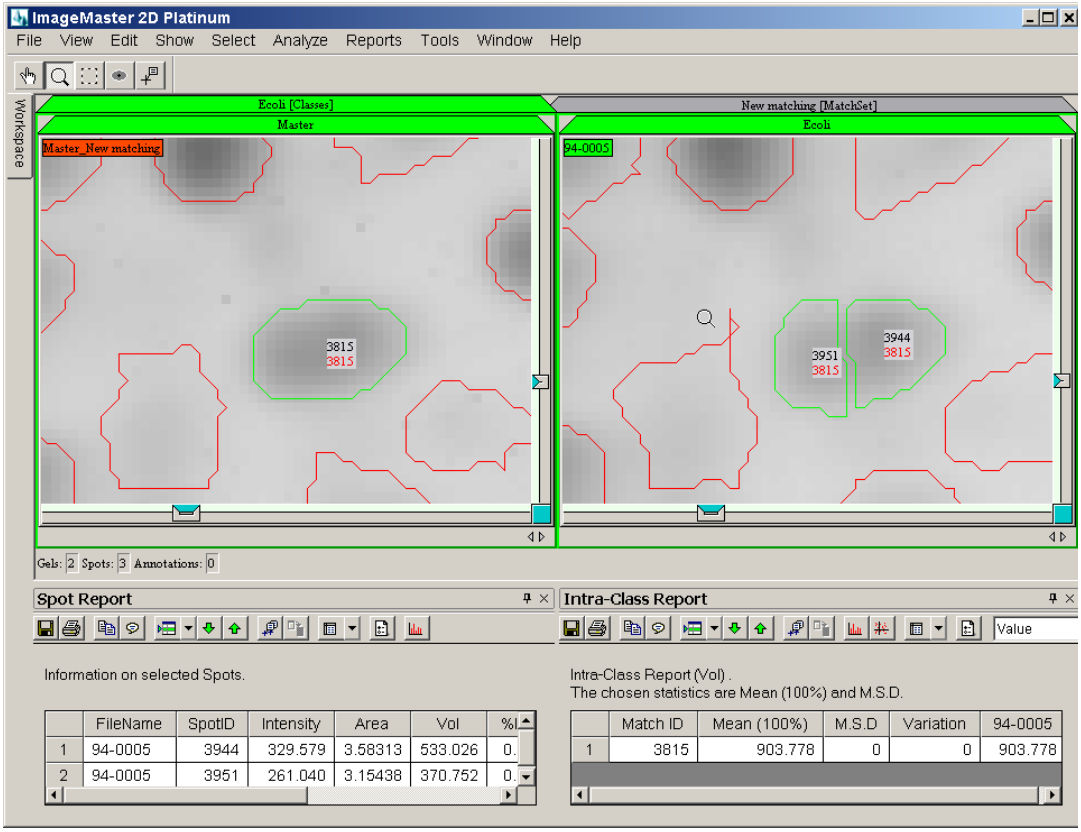


Figure 9-3. Multiple matches. One spot in the master gel (spot 3815) is matched with two spots (spots 3944 and 3951) in another gel. In further data analysis and quantification (e.g. Vol in the Intra-Class Report), the two spots are considered as being one (the volumes have been summed).

9.3.2 Reports

You can select matches directly from a displayed Match Report (see Section 9.6.1). You can do this by double clicking on a row in the report, or by selecting one or more rows and clicking the *Select on Gels* icon in the report toolbar.

9.4 Displaying matches

A match describes the relationship between individual spots. As mentioned above, selecting matches corresponds to selecting their constituent spots. However, this is not always a straightforward way to visualize the correlation

between spots. ImageMaster therefore provides the following features for easier visualization of matches.

9.4.1 Show / hide matches

Matches can be displayed as blue vectors that link the locations of the spots in the displayed gel and the matched spots in the master image (Figure 9-4). These match vectors are always minimized with respect to the center of the visible gel area and enable you to evaluate the matching results more easily.

You can change the default color for the match vectors by choosing *Tools* > *Options* from the menu and going to the *Display* tab.

To display match vectors:

1 Open a [MatchSet] or [Classes] worksheet.

2 Choose *Show* > *Matches* > *Show Vectors* from the menu.

3 Blue vectors are shown between matched spots in the displayed gel and the master gel.

4 You can hide the match vectors again by choosing *Show* > *Matches* > *Hide Vectors*.

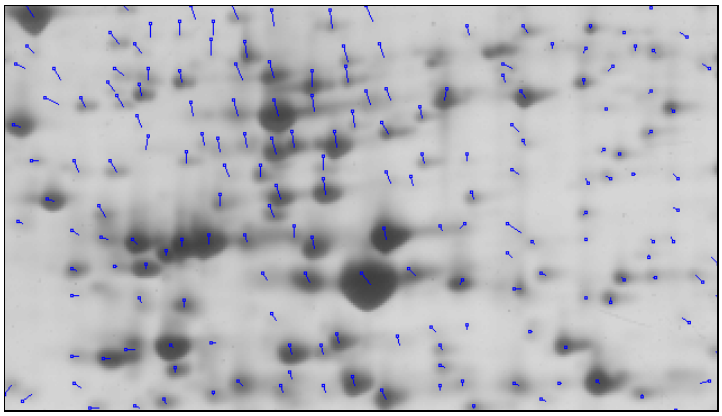


Figure 9-4. Matched spots in matched gels are linked by blue vectors.

9.4.2 Show / hide match IDs

You can display Match IDs for selected spots on selected gels (Figure 9-5). Obviously, the gels must be matched to the Master.

To display Match IDs:

1 Select the spots and gels you would like to display the Match ID for.

2 Choose *Show* > *Matches* > *Show ID* from the menu.

3 To hide the Match IDs again, possibly in specific gels only, select the desired gels and choose *Show* > *Matches* > *Hide All ID*.

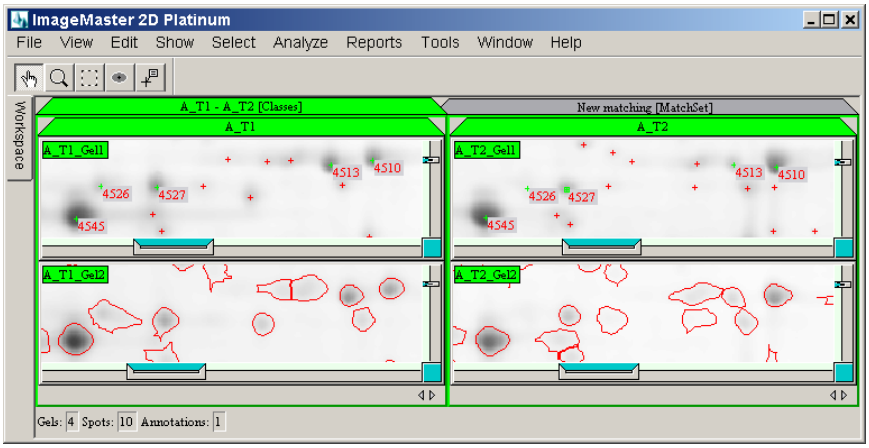


Figure 9-5. Match IDs for selected spots on selected gels.

9.5 *Adding / deleting matches*

Sometimes it may be necessary to manually add matches. This can be the case when the matching algorithm needs a few input matches, or when you would like to add or change matches after the automatic matching procedure.

9.5.1 Adding / deleting matches

To add a match:

1 Select a spot in the master image and the corresponding spots in the other images (you can select several spots in a given image if you want to create a multiple match). Use the Shift or Ctrl keys to make multiple selections.

2 Choose *Edit* > *Matches* > *Add Match* from the menu.

3 The new match is created.

To delete a match:

1 Select the spots for which matches should be deleted.

2 Select the gels from which the corresponding matches should be deleted. Use the Shift or Ctrl keys to make multiple selections.

3 Choose *Edit* > *Matches* > *Delete Match* from the menu.

4 The matches for the selected spots are deleted.

NOTE! To undo the addition or deletion of matches you should not only select the gel for which the operation should occur, but also the Master.

9.6 Reports on matches

9.6.1 Match report

ImageMaster can display a *Match Report*. It shows a list of matched spots in the selected gels. The first column in such a report displays the Match ID, that is, the Spot ID in the master image. The following columns display the corresponding Spot IDs in the matched gels that were selected for the creation of the report (Figure 9-6).

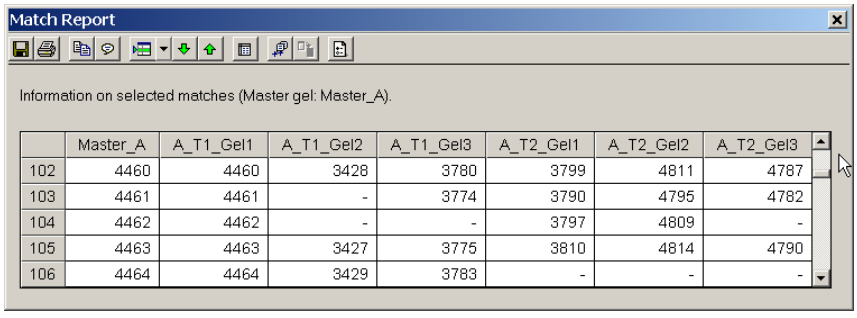
To display a Match Report:

1 Select the spots that should appear in the Match Report.

2 Select the matched gels you would like to include in the report.

3 Choose *Reports* > *Match Report* from the menu.

4 You get a list of spot IDs for all matched spots among the selected gels.



	Master_A	A_T1_Gel1	A_T1_Gel2	A_T1_Gel3	A_T2_Gel1	A_T2_Gel2	A_T2_Gel3
102	4460	4460	3428	3780	3799	4811	4787
103	4461	4461	-	3774	3790	4795	4782
104	4462	4462	-	-	3797	4809	-
105	4463	4463	3427	3775	3810	4814	4790
106	4464	4464	3429	3783	-	-	-

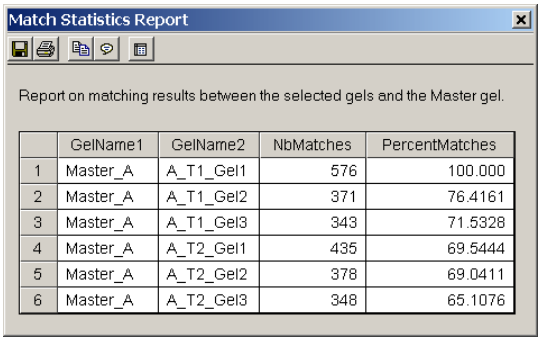
Figure 9-6. Report on selected matches for six matched gels.

9.6.2 Match statistics report

Another way to evaluate matching results is to generate a *Match Statistics Report* (Figure 9-7). This report lists the number of matches (**NbMatches**) found between each of the selected gels in the match set and the master image, as well as the percentage of spots that were matched (**PercentMatches**), calculated as follows:

$$\text{PercentMatches} = \frac{2m}{n_g + n_m}$$

where m is the total number of matches between the gel and the master image, n_g is the number of spots in the gel and n_m is the number of spots in the Master.



	GelName1	GelName2	NbMatches	PercentMatches
1	Master_A	A_T1_Gel1	576	100.000
2	Master_A	A_T1_Gel2	371	76.4161
3	Master_A	A_T1_Gel3	343	71.5328
4	Master_A	A_T2_Gel1	435	69.5444
5	Master_A	A_T2_Gel2	378	69.0411
6	Master_A	A_T2_Gel3	348	65.1076

Figure 9-7. Report on the match statistics between selected gels and the master image.

To display a Match Statistics Report:

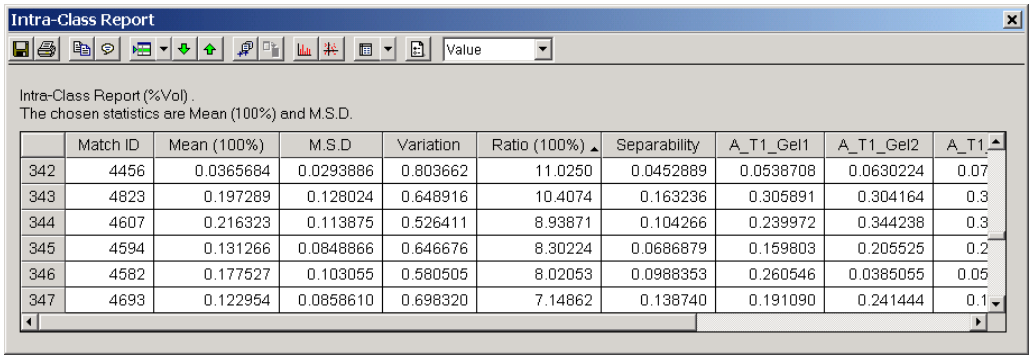
1 Make sure you opened a match set from the workspace.

2 Choose *Reports* > *Match Statistics Report* from the menu.

3 A *Match Statistics Report* is displayed.

9.6.3 Intra-class report

Intra-class Reports contain information about matched spots in a class of gels (Match ID, central tendency, dispersion, spot values, coefficient of variation, range ratio, separability, etc.). In Chapter 10 you can find in-depth information about the Intra-Class Report and its functionalities (Figure 9-8).



	Match ID	Mean (100%)	M.S.D	Variation	Ratio (100%)	Separability	A_T1_Gel1	A_T1_Gel2	A_T1
342	4456	0.0365684	0.0293886	0.803662	11.0250	0.0452889	0.0538708	0.0630224	0.07
343	4823	0.197289	0.128024	0.648916	10.4074	0.163236	0.305891	0.304164	0.3
344	4607	0.216323	0.113875	0.526411	8.93871	0.104266	0.239972	0.344238	0.3
345	4594	0.131266	0.0848866	0.646676	8.30224	0.0686879	0.159803	0.205525	0.2
346	4582	0.177527	0.103055	0.580505	8.02053	0.0988353	0.260546	0.0385055	0.05
347	4693	0.122954	0.0858610	0.698320	7.14862	0.138740	0.191090	0.241444	0.1

Figure 9-8. Intra-Class Report on matched spots in selected gels.

9.7 Synthetic gels

With the *Create Synthetic Gel* feature, you can create a composite gel image that contains only representative spots of a population, or on the other hand shows all spots present in a series of gels. In the first case, an average gel image is obtained by averaging the positions, shapes, and optical densities of the matched spots in a given set of gels. This produces an intersection of all gels, showing only the spots found in all or at least some of the given images. In the second case, the unmatched spots are added as well. The synthetic gel can then be considered as a spot index or a graphical fusion of protein maps.

In reality, you can choose which spots should be included in a synthetic gel. This is because the synthetic gel will contain all selected spots in the selected gels at the moment you create the synthetic image. You can therefore filter your spots carefully before generating the synthetic image.

If spots are matched, only one of the matched spots will be present in the synthetic gel. Its area corresponds to that of the spot having an area closest to the average of the matched spots, while the intensity equals the average intensity of the matched spots. For isolated spots included in the synthetic gel, the Intensity, Area and Vol values of the original spot are assigned. The %Vol and %Intensity values are recalculated according to the total Vol and Intensity of the new synthetic image.

The positions of the spots in the new synthetic gel are based on the positions in the master image, and therefore the reference image, of the match set. The resulting synthetic gel normally looks similar to the reference image.

NOTE! The spots from the reference gel image should be selected as well if the synthetic gel to be created should take into account the quantification values of this gel. Therefore, remember to display the reference gel in the match set by choosing *Show* > *Show Reference*, and to select the spots to be included in the synthetic image. The Master of the match set does not contain quantification information and will only be used for determining the spot positions in the new synthetic gel.

9.7.1 Spot filtering

Synthetic gels are created based on selected spots in selected gels. In order to obtain the cleanest possible synthetic gels, without artifacts or insignificant spots, you can refine your spot selection using the various filtering options in ImageMaster. You can perform a type of filtering that consists of selecting, and possibly eliminating, spots that do not satisfy certain criteria of size, volume, and so on.

To apply a spot filter:

1 Select all gels to be filtered.

2 Select all spots on the selected gels.

3 Choose *Select* > *Spots* > *Refine by Value* from the menu.

4 You are asked for the value type to be utilized for filtering, and the thresholds to be employed. Click *OK* when you finish entering the latter.

5 Only spots meeting the specified conditions are kept.

6 Depending on the applied filter, you can continue to use the current selection, or on the contrary, delete the selected spots or exclude them from your analysis (by hiding them, for instance).

A second filter can be applied. You can select matches (that is, the spots in the matches) that are representative of your population. Spots that are only present in one of five gels of a match set are not representative of the match set and should be excluded from the analysis. You can select matches by the number of spots they contain. For example, select all matches that have at least 4 (or 5) spots. In other words, you can select spots that are present in at least 4 (or 5) gels.

To apply a match filter:

1 Make sure the gels have been matched.

2 Select all gels for which a match selection should be refined.

3 Select spots or matches (can be the result of a previous spot filter).

4 Choose *Select* > *Matches* > *Refine Selection* from the menu.

5 Enter the threshold for the refinement of matches (see Section 9.3.1). For instance, you might **Keep the Matches whose number of selected spots is >=** 4 (or 5).

6 The remaining matches satisfy the specified criterion. You can obtain all spots that do not satisfy this criterion by choosing *Select* > *Spots* > *Inverse Selection*.

NOTE! You should check your results carefully. Detection or matching problems may cause the exclusion of spots that are actually present in all gels, or the inclusion of spots that are only present in one or two gels (but incorrectly matched to other spots). Moreover, when selecting matches, the program only takes into account the spots that are present in the master gel. Any spots absent in the Master are not considered, even if they are present in all other gels.

9.7.2 Creating synthetic gels

Once the spots on your gels have been filtered and only the spots of interest are selected, you can create a synthetic gel.

NOTE! Synthetic gels can be created from a [MatchSet] or [Classes] worksheet. When you work in a [Classes] worksheet, the used Master will correspond to the first common node in the match set structure and the synthetic gel will automatically be inserted at this level.

To create a synthetic gel:

1 Select the spots and gels to be included in the synthetic gel (see above).

2 Choose *Tools* > *Gels* > *Create Synthetic Gel*.

3 Specify the destination folder and file name in the *Create Synthetic Gel* window.

4 The synthetic gel is created (Figure 9-9) and saved, together with the automatically generated matches (between the synthetic gel and the master gel).

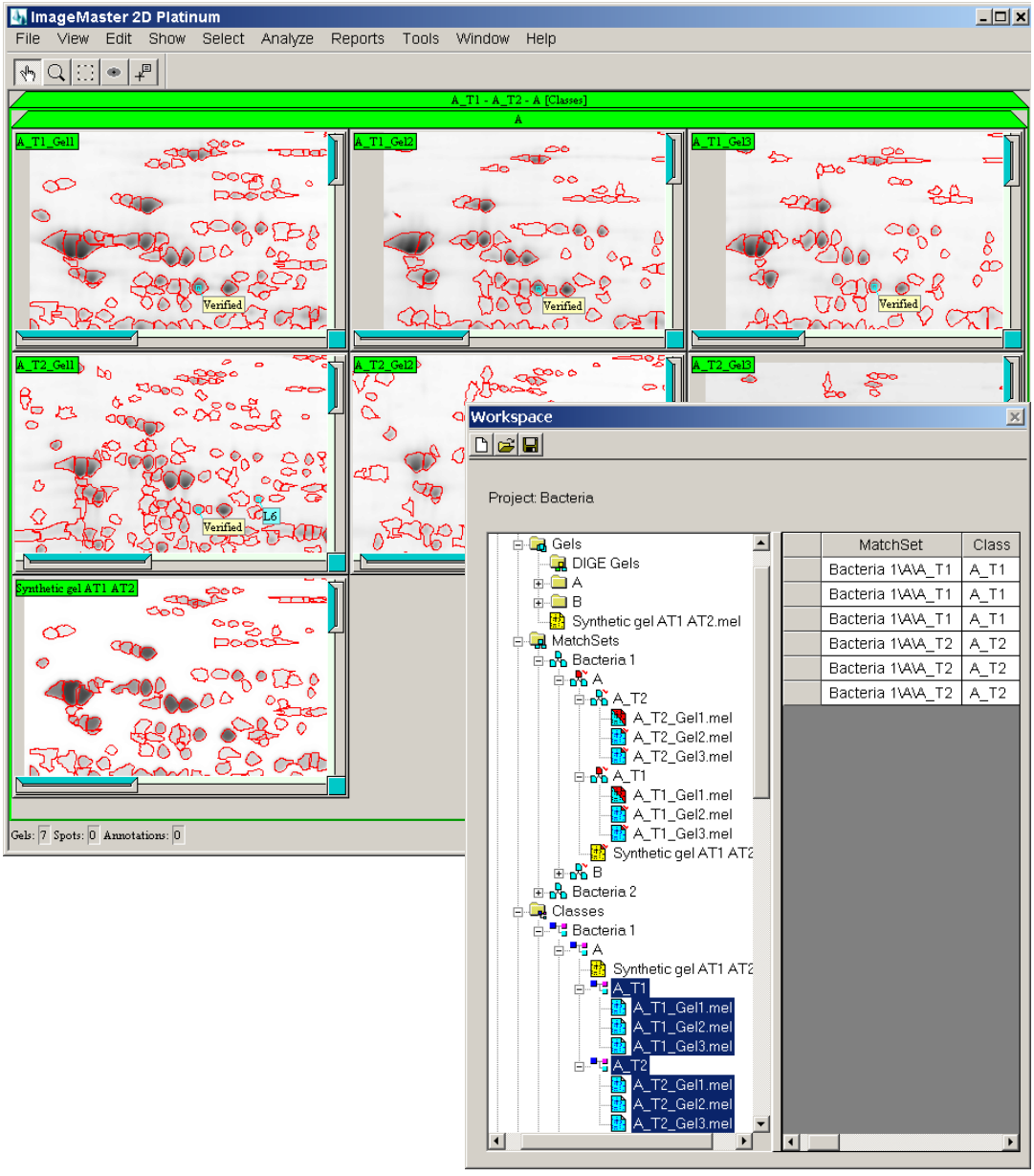


Figure 9-9. A synthetic gel (Synthetic gel AT1 AT2) and its six contributing gels. The classes A_T1 and A_T2 were originally opened in a worksheet to create the synthetic gel. Therefore, Master_A was used for the matching (first common node in the match set structure, see File Details) and the synthetic gel was automatically inserted at this level (in match set A).

10 Data analysis

10.1 Introduction

To study the variations in protein expression among a series of gels, the gels should be matched together (be part of the same match set), placed in classes, and opened in a [Classes] worksheet. Please refer to Chapter 4 to learn more about the workspace and the definition of classes.

Data analysis can be carried out at two different levels. Protein expression changes within a class of gels are studied with the **intra-class** statistics tools. These tools include:

- **Scatter plots**, to analyze gel similarities or experimental variations.
- **Descriptive statistics of central tendency and dispersion**, which can be calculated and displayed in Intra-Class Reports and Histograms.
- **Factor analysis**, enabling the identification of similar gels, and of spots that are characteristic for a particular population of gels.
- **Heuristic clustering**, an artificial intelligence technique to automatically classify sets of gels and highlight significant protein spots.

ImageMaster can also help find significant protein expression between classes of gels. For this purpose, two complementary statistical methods for **inter-class** analysis are available:

- **Overlapping measures**, which summarize each class of gels as an interval and measure the overlap between these intervals. The overlapping values can be displayed in Inter-Class Histograms and Reports.
- **Statistical tests**, which assess the difference between populations and give an estimate for the significance of the difference (that is, how high is the probability that this difference is only due to chance).

NOTE! The statistical tools in ImageMaster include all the options that the expert user might expect. Nevertheless, those who are less familiar with the statistical methods can simply use the default settings. Whenever possible, this manual indicates how the statistical data can be used and what precautions should be taken into account. However, keep in mind that the results are generally only indicative, and should always be checked carefully.

NOTE! For all data analysis functionalities described in this chapter, you must choose the spot quantification value on which the calculations should be based. You can choose between Intensity, Area, Volume, %Intensity, and %Volume. In addition, the Saliency is available for gels detected with the ImageMaster algorithm, and the Volume Ratio and Slope for spots detected with the DeCyder co-detection algorithm. For conventional 2-DE gels, the %Volume or Volume are generally indicated. For DIGE gels, the Volume Ratio should be selected in order to use the Internal Standard.

10.2 Intra-class statistics

10.2.1 Scatter plots

In order to analyze gel similarities or experimental variations such as disparities in stain intensity or sample loading, one can produce Scatter Plots for matched spots (Figure 10-1). Scatter plots give an idea of the relationship between the spot values (for example Intensity, Vol and %Vol) from two gels by searching for the linear dependence between the spot values of one gel (variable X) and the corresponding values in a reference gel (variable Y).

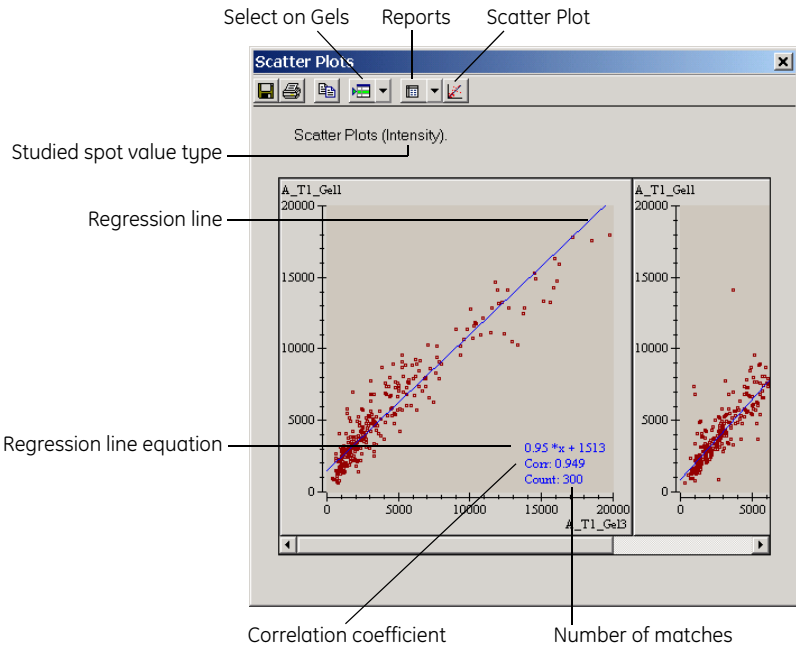


Figure 10-1. Scatter graphs for matched spots.

The linear dependence is defined as the best-fit line through the data points . The best-fit line is described by a slope and its offset from the equation **y = slope × x + offset**.

The goodness-of-fit for this approximation is given by the correlation coefficient **Corr**. This coefficient can vary between -1 and 1, where an absolute value near 1 indicates a good fit (the spot values of one gel can be predicted, to some extent, by the values of the other gel) while a low value indicates that the data could not be approximated by a straight line.

The types of conclusions that can be drawn from the regression line equations and the correlation coefficients:

1.0 × x + 0	and Corr = 1	indicates that the spot values for all matched spots are the same in the two gels.
1.2 × x + 0	and Corr = 0.95	indicates that almost all spot values are about 20% higher in the reference gel.
1.0 × x + 0.2	and Corr = 0.95	indicates that almost all spot values are shifted by +0.2 with respect to the reference gel.

In general, when the data are highly correlated (Corr close to 1) but the best-fit line is far from identity (1.0 × x + 0), you should check for possible reasons to explain why your values are systematically biased. Stain intensity variations, differences in protein loading or image acquisition problems are examples of typical causes.

To display scatter plots:

1 Make sure your gels are opened in a [Classes] worksheet.

2 Select a reference gel and the gels for which a scatter plot should be displayed.

3 Select matched spots to be included in the scatter plots. Generally, all matched spots are selected.

4 Choose *Analyze* > *Intra-Class* > *Scatter Plots*.

5 Select one of the spot value types (Intensity, Vol, Area, %Intensity, %Vol, Vol Ratio) to be plotted.

NOTE! To show the correlation between the spot quantities in the gels, the Intensity, Vol or %Vol values are most indicated, both for conventional 2-DE gels and DIGE gels.

The *Scatter Plots* window shows a scatter plot for each selected gel versus the specified reference gel, together with the best fit line, correlation coefficient and the number of matches displayed. If only one gel is selected (other than the reference gel), a single scatter plot is drawn. This scatter plot is completely resizable when the *Scatter Plots* window is in floating mode. You can drag the corners or borders of the window to make it smaller or larger. Selection of two or more gels other than the reference gel displays two (or more) scatter plots in one window. In this case, all scatter plots will have a fixed size and use the same scales on the X and Y axes.

The scatter plots are interactive. You can click on the points representing the matched spots and select them on the gels and other open reports. Just select matched spots by clicking on the corresponding data points while using the Ctrl or Shift keys for multiple selections. To select all data points in a region, hold down the mouse button and then drag the cursor to define an area. Use the options under the *Select on Gels* icon to identify the corresponding spots on the gels and open reports. You can also display a new scatter plot showing only the selected data points by clicking on the *Scatter Plot* icon.

As usual, you can save and print the graphics using the corresponding icons in the *Scatter Plots* window toolbar or copy plots to the clipboard for use in other applications. Information related to the scatter plots can be found in the *Reports* menu icon in the toolbar (Figure 10-1):

- The **Gel Report** gives information on the gels used in the scatter plots.
- The **Fitting Report** displays the slope and offset values of the regression line, the correlation coefficient and the number of data points (matched spots) for each plot.
- The **Scatter Report** displays the corresponding spot values in the gels and the error in relation to the regression line for each pair of matched spots. This error can be used, for example, to verify abnormalities between matched spots.

10.2.2 Descriptive statistics

Statistical analyses of the spot values within a class of gels allow you to analyze variations in protein expression among gels. ImageMaster provides the most commonly used descriptive statistics of central tendency and dispersion, which can be calculated and displayed in the Intra-Class Reports and Histograms.

These descriptive statistics are in fact numbers that summarize the spot values from a match. The central tendency allows you to localize the central value representing the data, whereas the dispersion indicates how closely the data points fall around the center. Based on these measures you can isolate matches composed of spots whose quantification values are unusual. The detected variations can be due to an inadequate detection or matching operation, or can result from protein expression changes among gels. Therefore, these statistics are not only useful for analyzing extracted data, but also for verifying process.

The descriptive statistics can be chosen with the following interface (Figure 10-2):

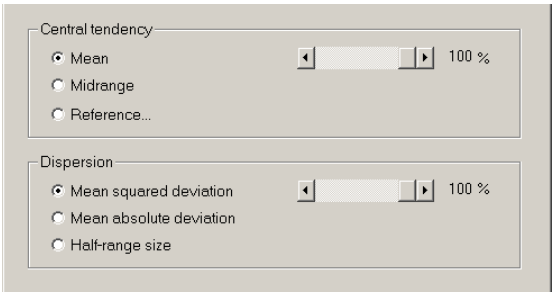


Figure 10-2. Statistics common to the Intra-Class Report, Intra-Class Histograms, Inter-Class Report and Inter-Class Histograms.

Central tendency

The central tendency gives the general location of a variable. This is commonly calculated by the arithmetic **mean** (also known as the average or center of gravity of a distribution), the **median** (the middle value which divides the sample in two equal parts) or the **midrange** (middle location between the two sample extremes).

Mean (or midrange) values are very sensitive to extreme values (outliers) and can be seriously contaminated by a single observation. On the other hand, the median is highly resistant to outliers. A compromise between mean (or midrange) and median is given by the **trimmed mean** (or midrange) where a predefined number of outliers are removed from the sample. The trimmed measures are more robust than the mean (or midrange) but are more sensitive than the median.

All of the above central tendencies can be obtained through the selections and the percentage slider available in the dialog box. The slider allows you to remove outliers. A 100% value means that all the spot values available in a match will be used to calculate the statistics (no outliers are suppressed). With a value of 80%, for example, 10% of the minimum values and 10% of the maximum values are discarded from the sample and the trimmed measure is calculated with the remaining values. Therefore:

- by just selecting **Mean**, the arithmetic mean is calculated, that is, the sum of all the sample values divided by the sample size.
- by just selecting **Midrange**, the midpoint of the sample value is calculated, that is, the middle location between the two sample extremes.
- to obtain a **Trimmed Mean** (or Midrange), you should select Mean (or Midrange) and discard the desired percentage of outliers with the percentage slider.
- to obtain the **Median**, you should select Mean and discard 50% of the values at each of the extremities, that is, select 0% in the percentage slider.
- by selecting **Reference...**, the value from the spot in a specified reference gel is taken as the central tendency.

Dispersion

The dispersion measures the variability of the sample data as indicated by how clustered or scattered the data points are around their center value. There are numerous measures of variability: standard deviation, range, interquartile range, and many others.

Like the statistics for central tendency, these measures make use of all the available sample data and can be heavily influenced by outliers. Therefore, you can also restrict the sample to the central values by trimming out the extreme values with the percentage slider.

- by selecting **Mean Squared Deviation** (M.S.D.), the square root of the average squared difference of each sample value to the center location is calculated.
- by selecting **Mean Absolute Deviation** (M.A.D.), the mean of the absolute difference between each value and the central value is calculated. It is not affected as much by outliers as the Mean Squared Deviation since the differences are not squared.
- by selecting **Half-range Size**, the difference between the largest and the smallest values divided by two is calculated.

NOTE! Notice that absent spots, represented by zero values, are also considered in the calculation of statistics (for both central tendencies and dispersion).

Examples

- The Mean 100% and the Mean Squared Deviation 100% are the most commonly used statistics (Figure 10-3, a). Notice that the standard deviation

is the Mean Squared Deviation multiplied by N, where N is the sample size. This difference comes from the fact that the standard deviation should be an unbiased estimator.

- The Median (Mean 0%) and Mean Absolute Deviation 100% are much more robust to outliers than the statistics above (Figure 10-3, b).
- The Midrange 100% and Half-range 100% define an interval that includes all sample values (Figure 10-3, c).
- The Midrange 50% and Half-range 50% are known as order statistics and interquartile ranges (Figure 10-4).

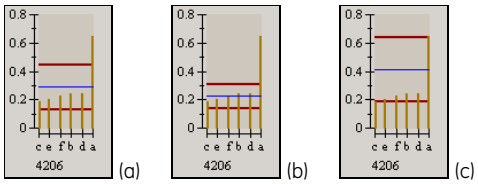


Figure 10-3. Histograms showing the sensitivities of central and dispersion values. (a) Mean 100% and M.S.D. 100%, (b) Median and M.A.D. (c) Midrange 100% and Half-range 100%.

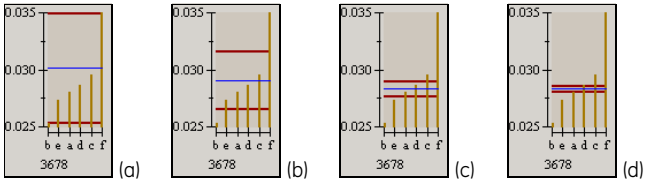


Figure 10-4. Histograms showing the effect of suppressing outliers. Midrange and half-range values are given for (a) 100%, (b) 80%, (c) 50% and (d) 33%.

10.2.3 Intra-Class Histograms

Intra-Class Histograms and Reports provide valuable tools for looking at unusual matches within a class. Analyzing protein expression changes, checking spot detection or verifying matching operations are just a few of the numerous potential usages. One can also compare spots visually or even navigate through matches. At this point, it should be noted that Intra-Class Histograms and Reports are dual elements; they have a reciprocal relationship. They are not only complementary tools to interpret the information, but are also designed to effortlessly switch between them. Starting from an Intra-Class Report you can easily display the corresponding histograms and vice versa. Histograms are a more visual way to look at matched spots.

To display Intra-Class Histograms:

1 Select the matches to be studied (on the gels or on an Intra-Class Report).

2 Select the gels to be included in the histograms. The gels can belong to different classes, but the class information will be ignored for intra-class statistics.

3 Choose *Analyze* > *Intra-Class* > *Histograms*.

4 In the pop-up list, select the value type (Intensity, Area, Vol, ...) to be displayed.

5 Choose the desired statistics (central tendency and dispersion values, see Section 10.2.2) in the subsequent dialog box.

Figure 10-5 shows histograms for some selected matches. The sliders at the bottom and the right side of the window allow you to see other matches. If many matches are selected, you may have to use the little page slider at the bottom right corner to see the matches displayed on additional pages.

In the histograms, the vertical orange bars correspond to the spot values, the blue horizontal line represents the chosen central tendency and the red lines delimit the range defined by [**Central value - Dispersion**, **Central value + Dispersion**]. The Match ID displayed at the bottom of each histogram allows you to keep track of the matches you are analyzing.

The histograms can of course be selected as with any other ImageMaster object and saved, printed or exported to other software (by using the corresponding icons). To select a histogram, simply click on it. To select many histograms, use the Shift or Ctrl keys.

After having selected the corresponding histograms, you can also re-select matches on the gels by using the *Select on Gels* icon. Navigating through the matches is accomplished using the *Select Next* and *Select Previous* buttons. Other selection possibilities (for example, *Select on Gels + Reports*, *Select from Gels*) are associated with the *Select on Gels* icon. Please see Chapter 5 for their detailed description.

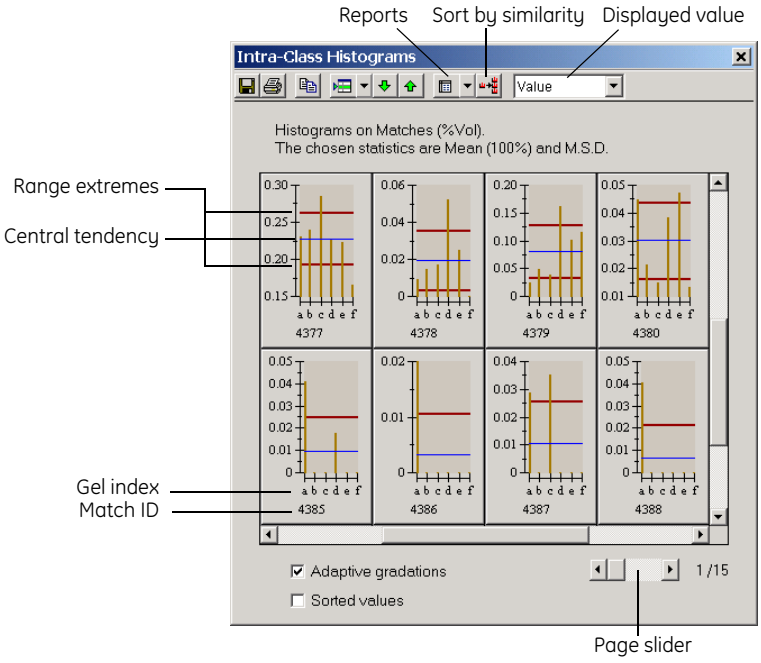


Figure 10-5. Intra-Class Histograms.

Additional information on the gels and matches is available from the *Reports* drop down menu in the toolbar (Figure 10-5):

- The **Gel Report** is a legend for the gel index on the histograms.
- The **Intra-Class Report** item displays the numerical values corresponding to selected histograms.
- The **Report from Selection** function creates a new histogram window that only contains the histograms that are selected in the active window.

NOTE! You can use the Intra-Class Report to change the order of your histograms (sort your data in the report and then re-display the corresponding histograms) or to refine your histogram selection (see Section 10.2.4).

In addition to the functions explained above, ImageMaster provides the following options in the histograms window to enhance the visual comparison of protein expression changes within matched gels.

Normalization

By default, the histograms display the raw spot values. Nevertheless, to simplify comparisons across matches, it can be convenient to normalize the spot values relative to their intra-class statistics. The following normalizations can be chosen from the *Displayed Value* list (Figure 10-5):

Value	Raw spot value.
Relative	|Spot value – Central tendency| This normalization places the central tendency values to 0, and if the Adaptive Gradations option (see below) is deactivated, you have a good overview of the dispersion and therefore of the homogeneity of the matches. This normalization is sensitive to high spot values.
Ratio	$\frac{\text{Spot value}}{\text{Central tendency}}$ This normalization divides all values by the central tendency and thus gives a ratio for all data. If you deactivate the Adaptive gradations option (see below), all histograms have the same scale and thus it becomes easier to detect matches that do not have homogenous values. This normalization is more sensitive to low spot values.
Normalized	$\frac{\lvert\text{Spot value} - \text{Central tendency}\rvert}{\text{Dispersion}}$ This normalization is a compromise between the two normalizations described above and is sensitive to all values.

Adapt gradations

To highlight spot variations inside a match, the histogram gradations are adjusted according to the spot values in each match. However, in order to display an identical gradation in all histograms, you can deselect the **Adaptive Gradations** check box at the bottom of the Histograms window (Figure 10-6).

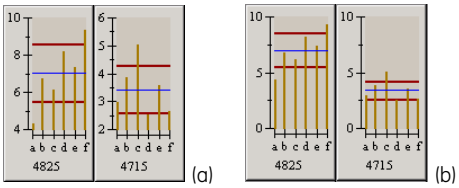


Figure 10-6. Histograms on matches with (a) adaptive gradations set individually for each histogram and (b) set according to the minimum and maximum values in all histograms.

Sort values

Additionally, to highlight and classify spot variations, you can display the spot values sorted in ascending order by clicking in the **Sorted Values** check box at the bottom of the Histograms window (Figure 10-7).

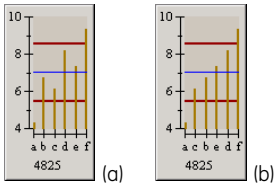


Figure 10-7. Histograms on matches with (a) unsorted values and (b) with values sorted in ascending order.

Sort by similarity

ImageMaster provides an easy way to look for similar histograms in the Intra-Class Histograms window. Select one histogram and click on the *Sort by Similarity* icon in the toolbar (Figure 10-5). A new histogram window is displayed, in which the selected histogram is depicted in the first position, and the other histograms follow according to a similarity criterion. This similarity criterion is based on the sum of the squared spot value differences for each gel G. The general formula is:

$$\text{Similarity} = \sum_{G=1}^{N} (v_i - v_j)^2$$

Where v_i is the spot value for match i, v_j is the spot value for match j, and N is the number of gels displayed in the histogram. The result generally is that histograms with similar shapes appear together (Figure 10-8). It must be noted that, when using raw spot values, very abundant spots predominantly influence the similarity criterion. To prevent this, it is better to use the *Sort by Similarity* option in combination with normalized spot values (see above).

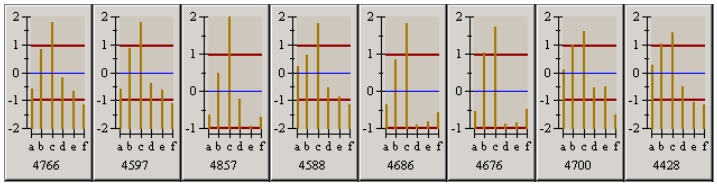


Figure 10-8. Histograms displayed according to their shape similarity.

10.2.4 Intra-Class Report

Similar to the Intra-Class Histograms, the Intra-Class Report describes numerical data from matches (i.e. their matched spots), as well as their corresponding

statistical values. In addition to the central tendencies and dispersion quantities described earlier (Section 10.2.2), other measures for the analysis of matched spots within a class of gels are available in the Intra-Class Report (Figure 10-9):

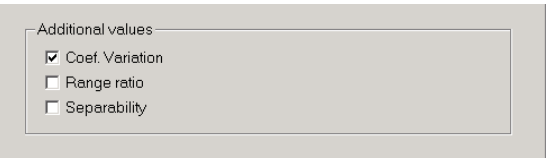


Figure 10-9. Additional values that can be added to the Intra-Class Report.

- The **Coefficient of Variation (Coef. Variation)** is the dispersion divided by the central tendency. It measures the relative variability of the spots in a match by correcting for the magnitude of the data values, thus giving a measure that has no units. When you choose the Median and Mean Absolute Deviation statistics, this measure is also known as the **Coefficient of Dispersion**.

- The **Range Ratio** is the maximum value divided by the smallest value in the sample specified by the percentage slider (used to suppress outliers from the calculated statistics, see Section 10.2.2).

- The **Separability** is the highest difference between two consecutively sorted values in the whole sample. It measures the greatest gap that you can have if you want to split the spot values in a match into two separate classes.

To display an Intra-Class Report:

1 Select a given number of matches (possibly all) to be studied.

2 Select the gels to be included in the histograms. The gels can belong to different classes, but the class information will be ignored for intra-class statistics.

3 Choose *Analyze* > *Intra-Class* > *Report*.

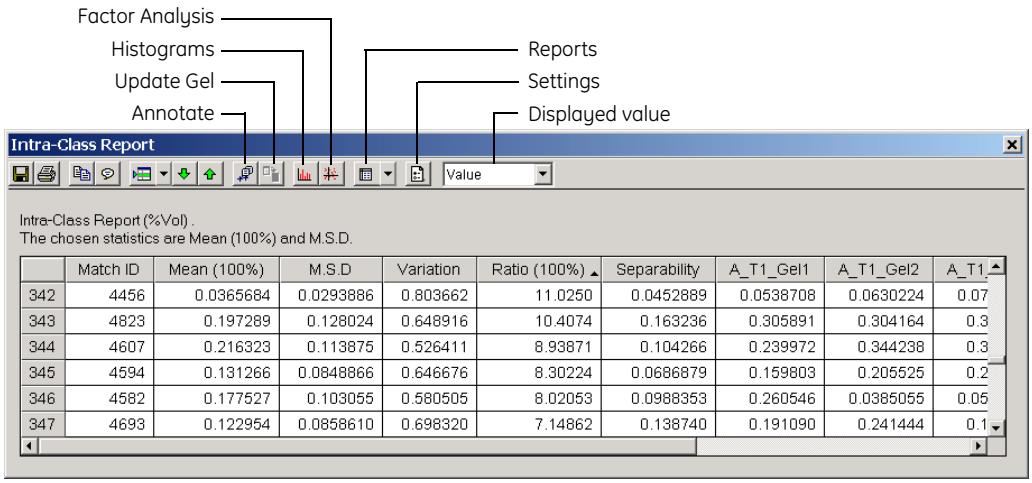
4 In the pop-up list, select the value type (Intensity, Area, Vol, ...) to be displayed.

5 Choose the desired statistics (central tendency and dispersion values, see Section 10.2.2) and the additional values to be calculated in the subsequent dialog box.

Figure 10-10 shows a typical Intra-Class Report. The sliders at the bottom and right side of the window allow you to see the rest of the table. The Match ID is followed by the desired statistical values, the coefficient of variation (optional), the

range ratio (optional), the separability (optional), and finally the spot values from each gel. These spot values can be normalized as described for Section 10.2.3, in order to simplify the comparison between matches. To do this, just select the desired type of normalization (Value, Relative, Ratio or Normalized) in the *Displayed value* list (Figure 10-10).

A number of standard functionalities are available from the Intra-Class Report toolbar (see Chapter 5 for details). Selected lines (use Shift or Ctrl keys for multiple selections) can be saved, printed or exported to other software. You can view your data on gels or related reports using the selection and navigation buttons. The Intra-Class Report supports the display and editing of annotation content, and therefore offers the *Annotate* and *Update Gel* functions. These functions enable you to add new categories and to propagate label modifications to your master gels. Finally, you can customize the content of the Intra-Class Report by using the *Settings* option.



	Match ID	Mean (100%)	M.S.D	Variation	Ratio (100%)	Separability	A_T1_Gel1	A_T1_Gel2	A_T1
342	4456	0.0365684	0.0293886	0.803662	11.0250	0.0452889	0.0538708	0.0630224	0.07
343	4823	0.197289	0.128024	0.648916	10.4074	0.163236	0.305891	0.304164	0.3
344	4607	0.216323	0.113875	0.526411	8.93871	0.104266	0.239972	0.344238	0.3
345	4594	0.131266	0.0848866	0.646676	8.30224	0.0686879	0.159803	0.205525	0.2
346	4582	0.177527	0.103055	0.580505	8.02053	0.0988353	0.260546	0.0385055	0.05
347	4693	0.122954	0.0858610	0.698320	7.14862	0.138740	0.191090	0.241444	0.1

Figure 10-10. Intra-Class Report.

By clicking on the *Histograms* icon in the toolbar (Figure 10-10), you can display the histograms of matches that were selected in the report. Please note that the order of the histograms in the resulting Intra-Class Histograms window will be the same as that of the selected matches in the Intra-Class Report. You can take advantage of this characteristic to organize your histograms in a certain way.

The *Reports* icon in the toolbar (Figure 10-10) allows you to display reports related to the matched spots:

- The **Gel Report** item is a reduced version of the original report on gels that can be used, for example, as a legend for the histograms.
- The **Spot Report** is a modified report on spots that is useful to compare one spot against the others in the same match (see the possible applications in the paragraph below).
- The **Report from Selection** function creates a new report that only contains the matches that are selected in the active report.

Spot report

In this report, the measures available for quantifying the spot values relative to their match are the same as above (Value, Relative, Ratio or Normalized). You can use these values to select spots that are outliers, or on the contrary, to select spots that are representative of a match:

Representative spots

- In order to select the most representative spots of a match, one could, for example, pick out the spots whose values are close to the mean (with less than 20% difference). To do this, choose the Mean 100% as central tendency, and then select all spots that have a Ratio (*Displayed value* list) between 0.8 and 1.2.
- Another possibility is to choose spots with a Median (Statistics) Ratio (*Displayed value* list) of 1, which will select the spots that are in the middle of the match.

Outliers

- Outliers can be selected by choosing values that are outside the range given by the mean ± two times the standard deviation. In fact, with a normal distribution, at most 5% of the values should be outside this interval. In practice, you can find such outliers by using the Mean (100%) and Mean Squared Deviation (100%), and then choosing all spots with a Normalized value (*Displayed value* list) higher than 2.
- When you calculate the median, and choose Ratio values (*Displayed value* list) larger than 2, you select spots that are at least 2 times higher or lower than half of the spots from the match.
- The Midrange (100%) and Half-range (100%) statistics, combined with a Normalized value (*Displayed value* list) of 1, allow you to select spots that are the highest or the lowest in their match.

- The Midrange (50%) and Half-range (50%), combined with a Normalized value (*Displayed value* list) superior to 2, will select spots that are at least 2 times higher or lower than 75% of the spots from the match.

NOTE! In order to restrict the number of selected matches, you can remove the matches that contain too many outliers. You can also use this functionality to search for unusual gels. If a gel contains more outliers than the others, it may be atypical or abnormal. You should determine the reasons for this abnormality before continuing the analysis.

10.2.5 Factor analysis

The visual task of comparing gels is rather difficult when dealing with a large number of gels that consist of thousands of spots. In particular, it can be hard to assess whether different sample populations exist and to characterize their different protein expression profiles. Factor analysis helps here, by finding a way to condense the information contained in such huge data sets into a smaller number of factors, or dimensions, that explain most of the variance observed. The factor analysis tool is used to examine the interrelationships between large numbers of variables (that is, spot values for a series of gels) and to explain these relationships (for example gel populations) in terms of common underlying factors (or associations with specific spot patterns).

Factor analysis is a complex statistical technique, whose comprehensive description is beyond the scope of this manual. For more information, please see Appendix C for references. In the following paragraphs, you learn how to perform a factor analysis and how to interpret the results. Your attention is also directed to some critical points of this analysis.

To carry out a factor analysis:

1 Click the *Factor Analysis* icon in the Intra-Class Report toolbar (Figure 10-10).

2 If any matches are selected, the program asks whether you want to use only the selected rows or not. Please see further on in this section to judge which of the options is most applicable.

3 The program displays a *Factor Analysis Report* with the lines corresponding to the axes that can be drawn in the Factor Projection Plot. Select 2 axes to be displayed in the plot (the first two ones, generally).

4 Click the *Plot Projection* icon in the Factor Analysis Report toolbar.

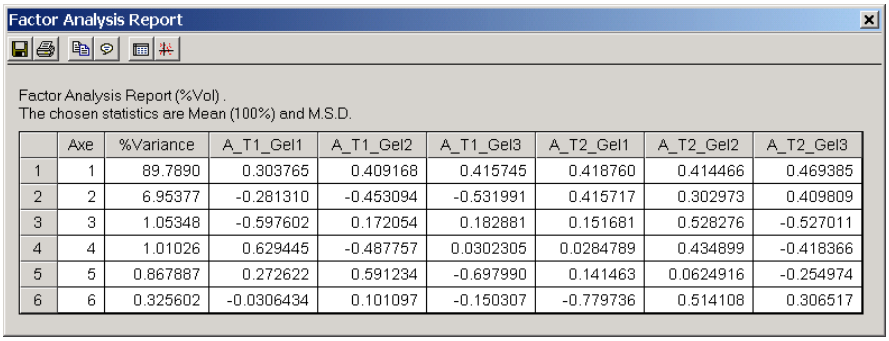
5 A dialog box lets you choose the number of matches to be displayed. Please note that the selected number does not influence the factor analysis results. It just improves the clarity of the plot by reducing the number of visible matches.

6 The *Factor Projection Plot* is displayed. It is completely resizable when in floating mode. You can drag the corners or borders of the window to make it smaller or larger.

Interpreting a factor analysis

The following explanations about how to interpret a factor analysis are illustrated with an example of six gels run to compare the effect of two treatments T1 (A_T1_Gel1, A_T1_Gel2, A_T1_Gel3) and T2 (A_T2_Gel1, A_T2_Gel2, A_T2_Gel3) on bacteria cultivated on substrate A. The gels were detected, matched and added to a class. The class was opened in a [Classes] worksheet, all the gels and matches were selected, and an Intra-Class Report was displayed.

The Factor Analysis Report (Figure 10-11) was obtained by clicking on the *Factor Analysis* icon in the Intra-Class Report toolbar. It summarizes the variance accounted for by successive axes (or factors), expressed as a percent of the total variance. Thus, factor 1 accounts for 83.7% of the variance, factor 2 for 7.5%, and so on. The report also lists the coordinates for each gel projected on these axes.


Factor Analysis Report

Factor Analysis Report (%Vol) .
The chosen statistics are Mean (100%) and M.S.D.

	Axe	%Variance	A_T1_Gel1	A_T1_Gel2	A_T1_Gel3	A_T2_Gel1	A_T2_Gel2	A_T2_Gel3
1	1	89.7890	0.303765	0.409168	0.415745	0.418760	0.414466	0.469385
2	2	6.95377	-0.281310	-0.453094	-0.531991	0.415717	0.302973	0.409809
3	3	1.05348	-0.597602	0.172054	0.182881	0.151681	0.528276	-0.527011
4	4	1.01026	0.629445	-0.487757	0.0302305	0.0284789	0.434899	-0.418366
5	5	0.867887	0.272622	0.591234	-0.697990	0.141463	0.0624916	-0.254974
6	6	0.325602	-0.0306434	0.101097	-0.150307	-0.779736	0.514108	0.306517

Figure 10-11. Factor Analysis Report.

The number of factors equals the number of gels being analyzed. Factor analysis cannot be performed with less than two gels and so at least two factors are always calculated. Of course, the more gels you use, the more reliable the results are likely to be, and the more factors will be calculated. Since the first factors are generally far more important for characterizing gels and matches than any subsequent ones, the factors are ranked in order of importance.

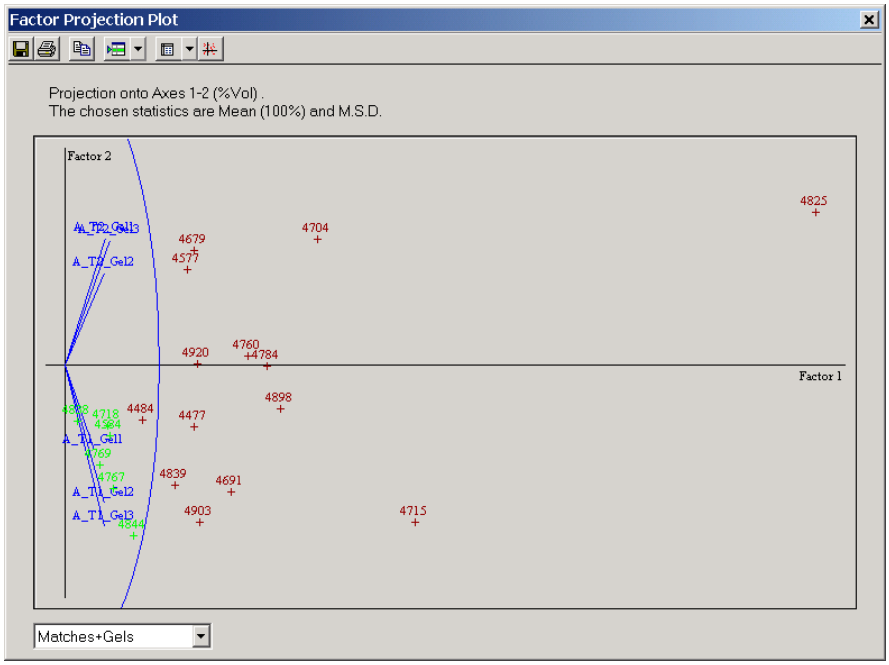


Figure 10-12. Factor Projection Plot. Note that the options in the lower left corner allow you to display only matches or only gels. The blue curve represents a part of the correlation circle; its form is linked to the scale of the axes. Points corresponding to matches can be selected on the plot (in green) and highlighted on the gels or any open reports.

In the current context of gel image analysis, it has been noted that the first two axes are usually the most significant ones for finding gels and matches that behave similarly. Figure 10-12 shows the Factor Projection Plot obtained when the first two axes in Figure 10-11 were selected. This plot displays the projection of each match (red or green cross) and each gel (blue vector) on the two factorial axes. In the present example, only the 20 most significant matches are displayed on the projection plot. If all matches were shown, one would find that many of them cluster around the origin of the graph. This illustrates that the majority of matches, i.e. protein spots, are not significant in classifying the gels. The further away a spot is from the origin, the more important it is likely to be in terms of characterizing the gels.

To find a possible meaning for a given factor, one should first identify the matches that largely contribute to this factor. The *Factor Projection Report* (Figure 10-13, a), available through the *Reports* item in the Factor Projection Plot, is used for this purpose. When sorting the matches in this report according to their contribution to the first axis, one discovers that matches 4825, 4715, 4704, 4898 and 4784

appear at the top of the table. Interestingly, these matches are those with the highest relative volumes, as found from the Intra-Class Report that is accessible in the Factor Projection Report toolbar. In fact, the first axis is generally correlated with protein abundance.

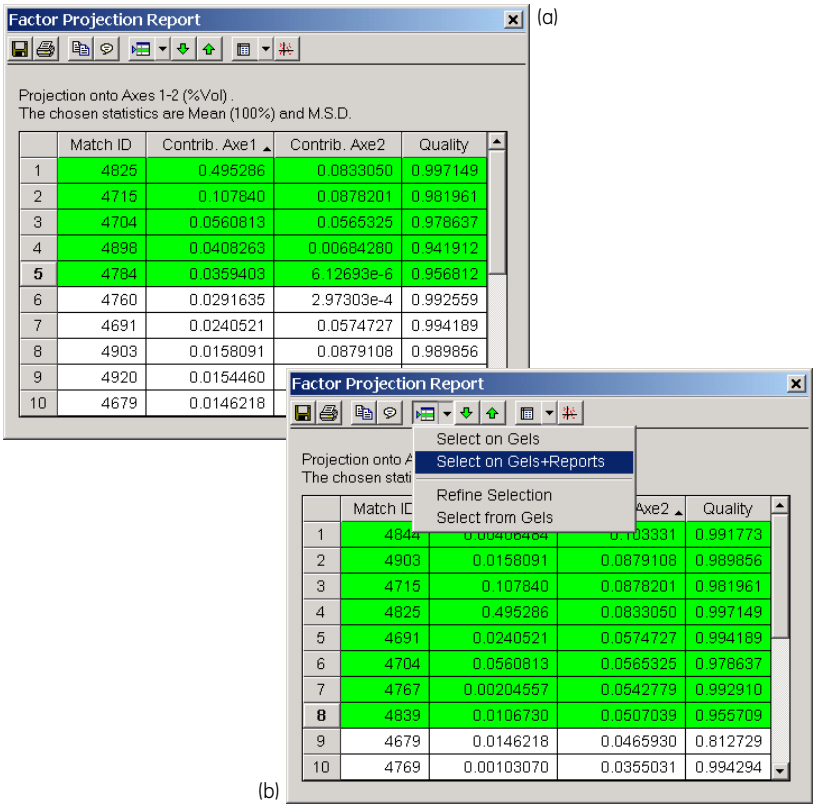


Figure 10-13. Factor Projection Report with (a) matches ranked based on their contribution to the first axis and (b) ranked based on their contribution to the second axis.

Please note that the Factor Projection Report also contains a Quality measure for each match. This number gives you an appreciation of whether a match is well represented on the factorial subspace. It tells you how close the distance of the projection is to reality. Indeed, matches with very similar behavior (similar expression profiles across gels) will be close in space. However, when projected onto a two-dimensional subspace, matches that are actually far apart may appear together. It is therefore important to look at the Quality measures to judge whether matches are effectively close. If the values are high for both matches, the chance is great that they are indeed nearby and have a similar behavior. If one of the matches has a low value, any interpretation becomes tentative.

Gels that are adjacent on the graph are likely to be similar to each other. They may correspond to the same population. This is clearly the case in the example above. The gels from T1 cluster together below the horizontal axis, whereas the gels corresponding to T2 lie above.

The closer a match is to a given set of gels, the more characteristic it is likely to be of those gels. That is, the more important the match is in determining why those gels are different from other gels. In Figure 10-12, for instance, one can observe that the matches 4844, 4903, 4691, 4767 and 4839 are close to the gels belonging to T1. The histograms in Figure 10-14 show that these matches have high spot values for the T1 gels and low values for the T2 gels. Match 4704, on the other hand, is characteristic of the T2 population, with higher spot values in the T2 gels. In our example, the second axis appears very important for separating the gels into two populations. It is related to the ratio between the mean spot values in each population of gels. The matches in the upper part of the graph have ratios that favor the T2 population, while those below the horizontal axis have ratios that favor the T1 gels.

The Factor Projection Plot is interactive. You can click on the points representing the matches and select them on the gels and other open reports. Just select matches by clicking on the corresponding points while using the Ctrl or Shift keys for multiple selections. To select all matches in a region, hold down the mouse button and then drag the cursor to define an area. Use the options in the *Select on Gels* drop down menu to identify the corresponding spots on the gels and open reports. You can also display a new factor projection plot showing only the selected matches by clicking on the *Plot Projection* icon.

You can save and print the graphics using the corresponding icons in the Factor Projection Plot window toolbar or copy the plot to the clipboard for use in other applications. Information related to the Factor Projection Plot can be found in the *Reports* drop down menu in the toolbar:

- The **Factor Analysis Report** summarizes the variance accounted for by the successive axes, expressed as a percent of the total variance. The report also lists the coordinates for each gel projected on these axes (Figure 10-11).
- The **Projection Report** displays the contribution of each match to the two axes displayed in the corresponding Factor Projection Plot, as well as a Quality measure that gives you an appreciation of whether the projection of the match is well represented on the factorial subspace (Figure 10-13).
- The **Intra-Class Report** for the selected matches, shows the individual quantification values for each spot in the match and allows to display related Intra-Class Histograms (Section 10.2.3).

Comments on factor analysis

Factor analysis is used to examine the protein expression pattern within each match. The quality of the factor analysis output depends on the quality of spot matching. Hence, it may be useful to exclude spots that are not well matched across all gels (using the *Select* > *Matches* > *Refine Selection* function from the menu, as described in Section 9.3.1). Nevertheless, in cases where a majority of spots were properly matched, the inclusion of all matches in the factor analysis can yield good results with no preliminary match filtering necessary.

This statistical method, based on data variation and their standard deviations, highlights the natural formation of classes among the gels and allows identification of matches (i.e. matched spots) that are characteristic of these classes. However, one should be very critical when analyzing factor analysis plots, as the results can be greatly influenced by outliers, bad matches, and so on. Factor analysis can provide valuable indications in some cases, but not in others.

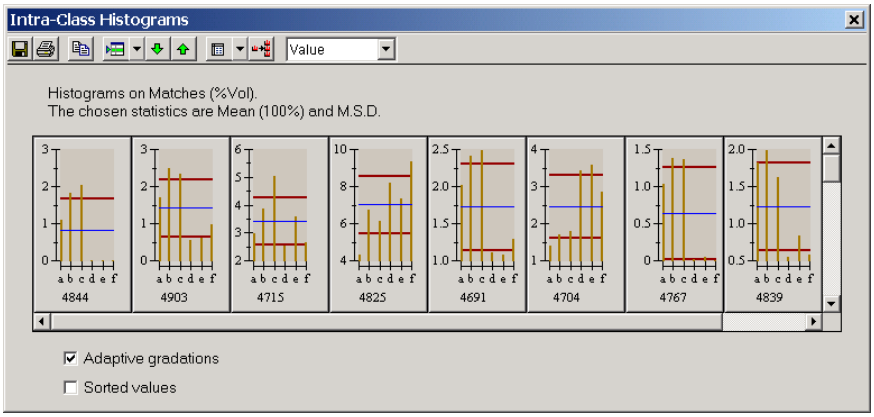


Figure 10-14. Intra-Class Histograms ranked based on their contribution to the second axis. Matches 4844, 4903, 4691, 4767 and 4839 are characteristic for population T1, match 4704 is characteristic for population T2. The gel legends are given in the corresponding Gel Report.

10.2.6 Heuristic clustering

ImageMaster proposes a powerful analysis method to automatically create classes of gels and highlight significant protein spots. Heuristic clustering, a machine learning algorithm based on artificial intelligence, is used to describe the characteristic spots of 2-D gels while using heuristics to speed up the search. The algorithm provides the possibility to blindly classify similar gels into two or more classes and to determine the characteristic spots (i.e. matches) of each class. These characteristic spots often correspond to differently expressed proteins.

The number of classes that are formed is dependent on the user input. ImageMaster first asks for the minimum and maximum number of classes, for example 2 and 4, respectively. It then splits the gels in 2, 3 and 4 classes, compares the resulting classifications and, according to a similarity function, shows the best one.

The classification step can also be repeated for each of the classes in order to find subclasses. In this way, a descending hierarchical classification tree is produced. The depth of classification, or the number of sublevels, is established by the **Classification Level** parameter that must be defined by the user.

The number of characteristic spots is determined by the **Sensitivity** parameter. This defines the gap between two classes (the difference between the highest spot value in one class and the lowest value in a second class, Figure 10-15). The smaller this parameter, the smaller the accepted difference between the class intervals in a match. Additionally, the number of spots considered in the classification is higher.

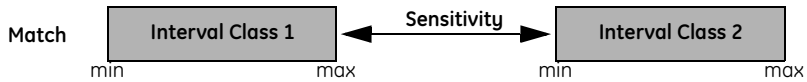


Figure 10-15. Sensitivity parameter.

To search for classes with the heuristic clustering algorithm:

1 Select the matches (possibly all) to be considered in the classification.

2 Select the gels to be classified. The gels can belong to different classes, but the class information will be ignored for the heuristic clustering.

3 Choose *Analyze* > *Heuristic Clustering* > *Do Clustering*.

4 In the pop-up list, select the value type (Intensity, Area, Vol, ...) to be considered.

5 In the subsequent dialog box, define the **Classification Level**, the **Minimum** and **Maximum number of classes**, and the **Sensitivity** parameter.

Depending on your computer capacity, this could take time, especially when the maximum number of classes is high, and many matches and gels were selected.

When the heuristic clustering process is finished, ImageMaster automatically creates default classes in the workspace and opens them in a new [Classes] worksheet. The matches that were found to be characteristic for one of the classes are shown in an *Inter-Class Histograms* window. You can learn more

about Inter-Class Histograms in Section 10.3.3. They display for each class formed, the central tendency (blue horizontal line) and the dispersion interval (red vertical line). To find out which gels compose each class (designated with a letter), you can select the *Gel Report* item from the *Reports* icon.

NOTE! The matches that stay selected on the gels after the heuristic clustering analysis are those that were considered significant in the analysis (and are therefore present in the Inter-Class Histograms).

Plot

The resulting classification can be visualized in a dendrogram, which represents the classes and their gels in a descending classification tree (Figure 10-16). To plot such a dendrogram, choose *Analyze* > *Heuristic Clustering* > *Plot*.

By using the icons from the toolbar in the *Heuristic Clustering Plot* window, the dendrogram image can be saved (in .png, .bmp or .tif format), printed, or copied to the clipboard for use in other applications.

10.3 Inter-class statistics

10.3.1 Specifying classes

To identify protein expression variations between populations of gels, you need to specify what gels belong to which population by creating classes. There are several possibilities for doing this. The first one requires that you already know the populations in your set of gels. This is the case, for example, when you are comparing gels from healthy tissue extracts with those from disease-associated samples. You can then define the classes yourself. Classes are created in the Workspace (see Chapter 4).

When you have no preliminary knowledge of the populations in the set of gels, you can implement a heuristic clustering analysis (see Section 10.2.6) and possibly draw conclusions from a factor analysis study (see Section 10.2.5). When doing heuristic clustering, classes are automatically defined, and you can analyze them with the tools described below.

10.3.2 Overlapping measures

As for the intra-class analysis, the spot values of each protein class can be summarized by two statistical descriptors, which are the central tendency and the dispersion (see Section 10.2.2). In addition to these descriptors, ImageMaster computes overlapping measures between the class intervals, which are defined by the ranges [**Central value - Dispersion**, **Central value + Dispersion**]. These ranges quantify the overlap between the spot values from two classes, and thus gauge how different the protein expression changes between the classes really

are. These measures of overlap take into account both the difference between the central tendency in each population and the dispersion.

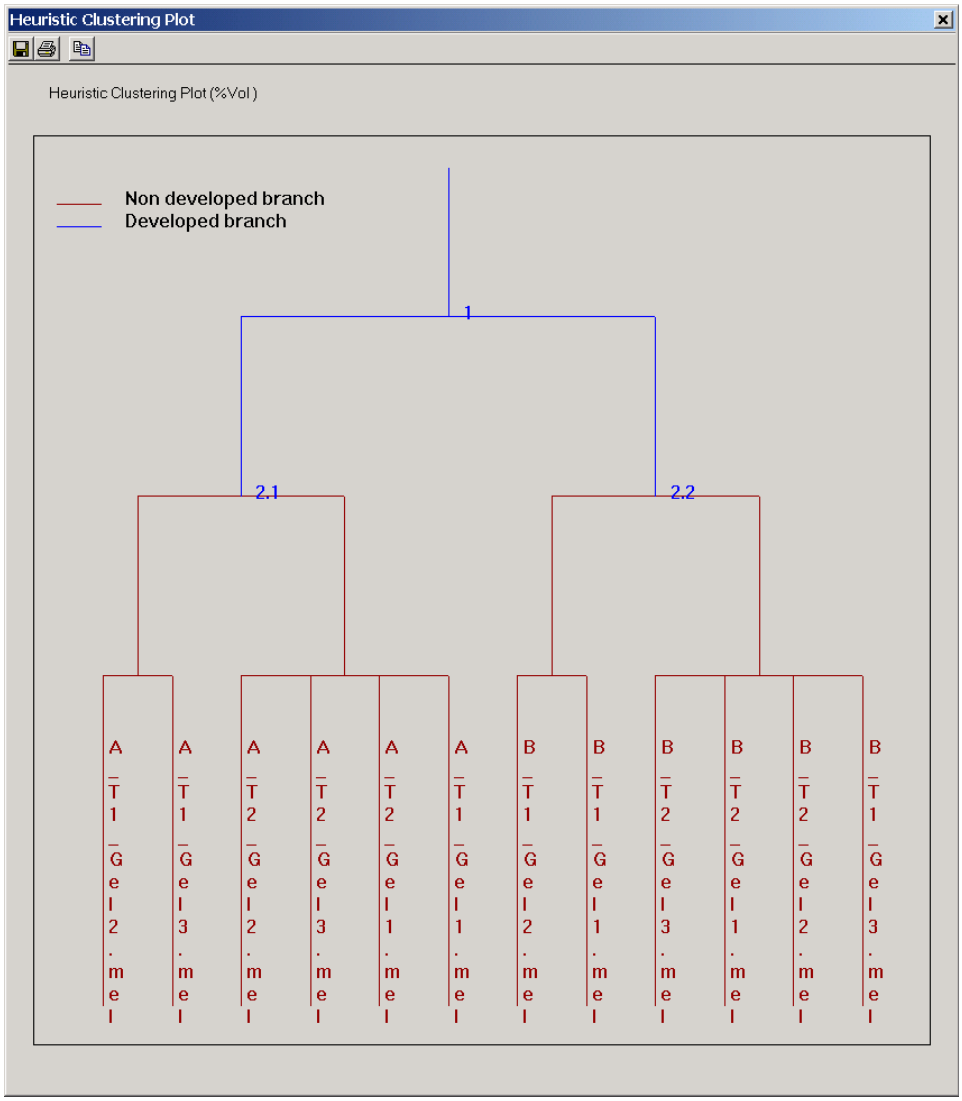


Figure 10-16. Dendrogram (with two classification levels) representing the classes found and their corresponding gels. In the example, gels were run on samples from bacteria grown on two different substrates (A and B), and for which two different treatments (T1 and T2) were tested. The substrate populations were found by the clustering algorithm. But the different treatments were not correctly recognized through heuristic clustering (each time a gel from T1 has been clustered with the gels from T2). This could be due to the way the match sets were defined though.

In the Inter-Class Histograms and Reports, the displayed statistical descriptor or overlapping measure needs to be selected from the *Displayed value* list in the toolbar:

- **Center**: Raw central tendency of the current class.
- **Dispersion**: Raw dispersion value of the current class.

> **NOTE!** The center and dispersion values define the interval that characterizes the protein sample of each class in a match. To characterize a class only by the central value (for the calculation of the difference or ratio between central values, for example) set the dispersion percentage slider to 0%.

- **Gap**: Maximum difference between the range of the current class and the range of one of the other classes (in the example of Figure 10-17, c-b in the case of Class A). Negative values indicate overlapping intervals whereas positive values are non-overlapping class ranges.
- **Ratio**: Maximum ratio between the lower limit of one of the other classes and the upper limit of the current class (in the example of Figure 10-17, c/b in the case of Class A). Absolute values smaller than 1 indicate overlap, whereas absolute values higher than 1 show that there is no overlap. In order to easily distinguish matched spots that are over or under expressed in one of the classes, the ratio value is preceded by a minus sign when the protein spot is under expressed for the class in question, compared to the other class. Positive values are attributed to the Ratio value in the over-expressed class.
- **Normalized**: Maximum percentage of the current class range not overlapping with the range of one of the other classes (in the example of Figure 10-17, (c-a)/(b-a) in the case of Class A). A value smaller than 1 indicates overlap. For example, 0.25 implies that 25% of the current class range is not recovered by one of the other classes. In the same way, a value of 1.5 indicates that there is a gap equivalent to 50% of the current class range to the furthest other class. The normalized overlapping is not symmetrical, the value from Class A compared to Class B is not the same as the value from Class B compared to Class A.

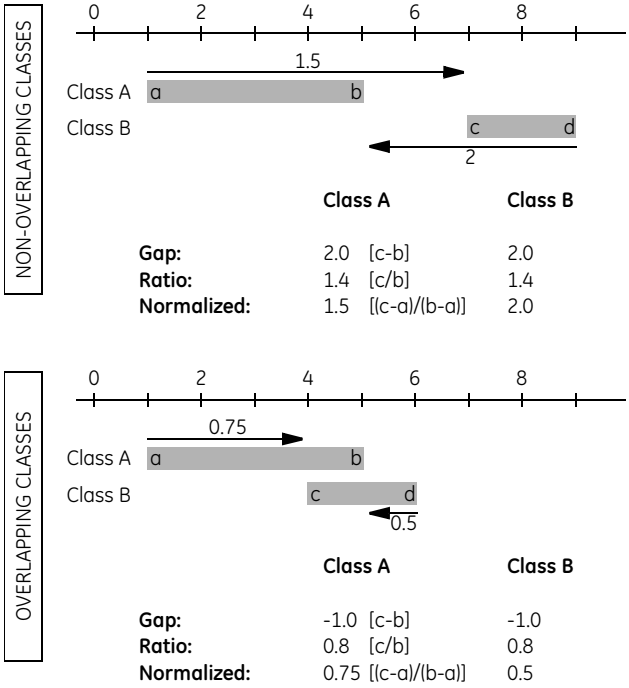


Figure 10-17. Scheme demonstrating how the Gap, Ratio and Normalized values are calculated for two non-overlapping classes (upper part) and two overlapping classes (lower part). Arrows above or below each class range illustrate how the Normalized measure relates to this class range.

Please note that the above mentioned formulas only apply to Class A for these particular examples. Their presence in this manual is only to illustrate the principles of the overlapping measures used in ImageMaster. Many different cases (and therefore formulas) exist.

Observe that in the Inter-Class Histograms and Reports the number 1000000 characterizes the cases where the protein is completely absent from a class (in this case ImageMaster cannot compute ranges). A value of 0 for the Ratio or Normalized measures indicates that the particular class is entirely covered by another one.

NOTE! For non-expert users, it is recommended to compare only two classes at a time in the Inter-Class Report (see Section 10.3.4). This is because only in this case one gets the direct relationships (difference, ratio or percentage overlap) between the two classes. As indicated in their definition, the Gap, Ratio and Normalized values always calculate the MAXIMUM difference, ratio or percentage, respectively, with respect to ANY of the other classes. This means that when the Ratio values for three classes (e.g. A, B, C) are compared, for instance, the software calculates the ratio of A with respect to B and of A with respect to C, but only displays the highest value in the column for class A. The number shown does not indicate with respect to which class the value was obtained. The idea is to quickly enable you to find a match (i.e. protein marker) that distinguishes the current class from any of the other classes. Once such protein markers are found, the complementary tools in the software, such as the Inter-Class Histograms (see Section 10.3.3), can be used to study the match in more detail.

10.3.3 Inter-Class Histograms

As with intra-class statistics, you can visually investigate the statistical and overlapping descriptors of classes by displaying Inter-Class Histograms.

To display Inter-Class Histograms:

1 Select the matches to be studied (on the gels or on a report).

2 Select the gels to be included in the analysis.

3 Choose *Analyze* > *Inter-Class* > *Histograms*.

4 In the pop-up list, select the value type (Intensity, Area, Vol, ...) to be displayed.

5 Choose the desired statistics (central tendency and dispersion values, see Section 10.2.2) in the subsequent dialog box.

By default, the displayed histograms (Figure 10-18) show the raw central tendency (blue horizontal line) and the dispersion interval (red vertical line) for each class. Visualizing the various overlapping measures is possible by changing the *Displayed value* in the list at the top of the *Inter-Class Histograms* window.

The Inter-Class Histograms can be selected as with any other ImageMaster object and saved, printed or exported to other software using the icons in the toolbar (Figure 10-18). You can also re-select the corresponding matches on the gels by using the *Select on Gels* drop down menu, and systematically navigate through matches with the *Select Next* and *Select Previous* icons. Finally, you can display more complete Inter-Class + Intra-Class Histograms (see below) for

selected histograms. These mixed histograms display the individual spot values in addition to the statistical descriptors for each class.

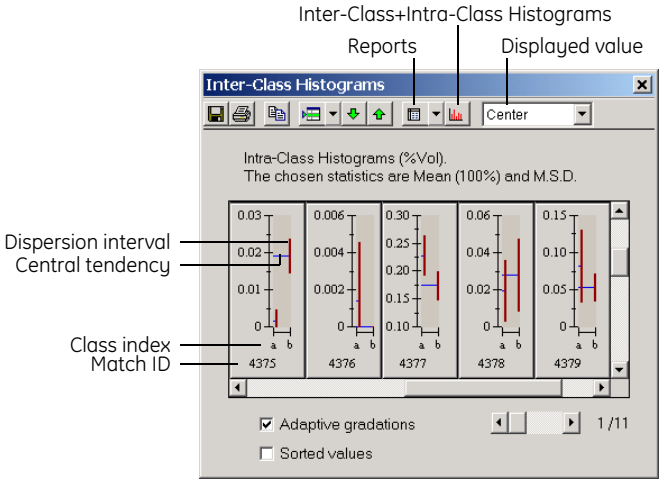


Figure 10-18. Inter-Class Histograms

The *Reports* drop down menu in the toolbar (Figure 10-18) allows you to display information related to the Inter-Class Histograms:

- The **Gel Report** item is a reduced version of the original report on gels to be used, for example, as a legend to the classes.
- The **Inter-Class Report** (see Section 10.3.4) lists the numerical values corresponding to the selected histograms.
- The **Intra-Class Report** displays a report on selected matches for a selected class. It is a reduced Intra-Class Report, where only the spot values of one class are considered. The information displayed is that which is usually found in an Intra-Class Report (see Section 10.2.4).
- The **Report from Selection** function creates a new report that only contains the lines that are selected in the active report.

NOTE! Analogous to the Intra-Class Histograms and Reports, the Inter-Class Histograms and Reports are dual elements; they have a reciprocal relationship. You can therefore use the Inter-Class Report to change the order of your histograms (sort your data in the report and then re-display the corresponding histograms) or to refine your histogram selection.

Adapt gradations

By default, the histogram gradations are adjusted according to the spot values in each match. However, in order to display an identical gradation in all histograms, you can deselect the **Adaptive Gradations** check box at the bottom of the histograms window (Figure 10-19).

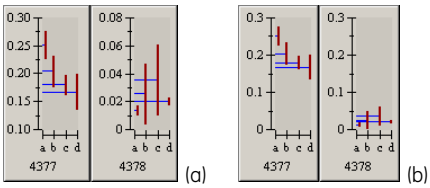


Figure 10-19. Inter-Class Histograms with (a) adaptive gradations set individually for each histogram and (b) set according to the minimum and maximum values in all histograms.

Sort values

To simplify the visual search for non-overlapping intervals, you may want to classify the displayed class values in ascending order, by clicking in the **Sorted values** check box at the bottom of the histograms window (Figure 10-20).

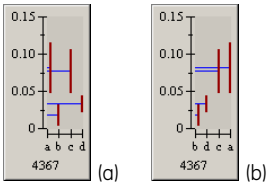


Figure 10-20. Inter-Class Histograms with (a) unsorted values and (b) values sorted in ascending order.

Inter-Class + Intra-Class Histograms

ImageMaster can draw more detailed Inter-Class Histograms for selected matches. These histograms not only display the class intervals but also show the individual spot values in each class. They can therefore be considered as a kind of mixed representation with properties from both the Intra-Class and Inter-Class Histograms.

To display Inter-Class + Intra-Class Histograms:

1 Select the matches to be studied (on the gels or on a report).

2 Select the gels to be included in the analysis.

3 Choose *Analyze* > *Inter-Class* > *Histograms*.

4 In the pop-up list, select the value type (Intensity, Area, Vol, ...) to be displayed.

5 Choose the desired statistics (central tendency and dispersion values, see Section 10.2.2) in the subsequent dialog box.

6 In the toolbar of the displayed *Inter-Class Histograms* window, click on the *Inter-Class+Intra-Class Histograms* icon.

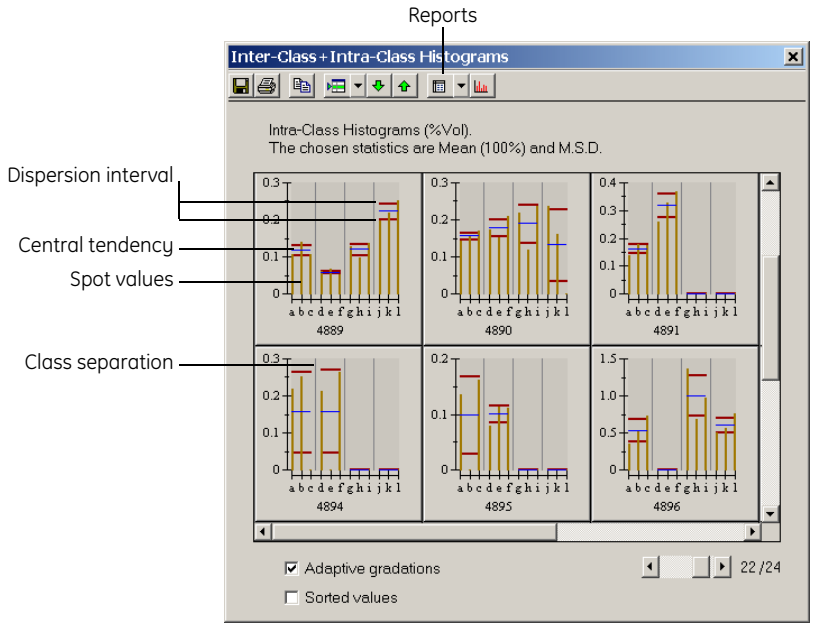
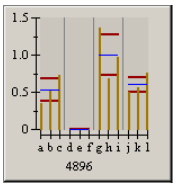
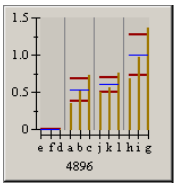


Figure 10-21. Inter-Class + Intra-Class Histograms.

As shown in Figure 10-21, the Inter-Class + Intra-Class Histograms display all the individual spot values in each match, separated for each class by vertical gray lines. The classes are characterized by their central tendency (blue horizontal line) and dispersion interval (bounded by the outer red lines).

The information, icons and functions enclosed in these specific histograms are similar to those described in the Intra-Class Histograms (Section 10.2.3) and Inter-Class Histograms (Section 10.3.3). The only point to be emphasized here is that selecting the **Sorted Values** check box at the bottom of the *Inter-Class + Intra-Class Histograms* window not only implies the sorting of the classes according to their central value but also the sorting of the spot values inside each class (Figure 10-22).



(a)


(b)

Figure 10-22. Inter-Class + Intra-Class Histograms with (a) unsorted values and (b) values sorted in ascending order. The classes are sorted according to their central value, and the spot values within each class are also sorted.

10.3.4 Inter-Class Report

ImageMaster allows you to generate specific reports in which you can numerically display one of the statistical descriptors or overlapping measures for each match and class, as well as the maximum value among all the classes.

To produce an Inter-Class Report:

1 Select the matches to be studied (on the gels or on a report).

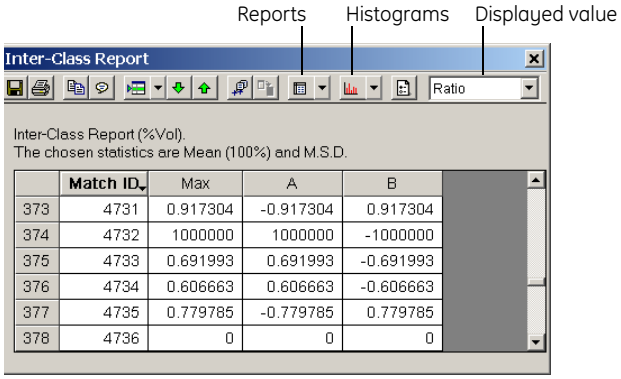
2 Select the gels to be included in the analysis.

3 Choose *Analyze* > *Inter-Class* > *Report*.

4 In the pop-up list, select the value type (Intensity, Area, Vol, ...) to be displayed.

5 Choose the desired statistics (central tendency and dispersion values, see Section 10.2.2) in the subsequent dialog box.

The Inter-Class Report (Figure 10-23) displays the descriptor selected from the *Displayed value* list for each class and gives the maximum value among all the classes (**Max Column**). It is thus possible to differentiate one class from the others according to a match.



	Match ID	Max	A	B
373	4731	0.917304	-0.917304	0.917304
374	4732	1000000	1000000	-1000000
375	4733	0.691993	0.691993	-0.691993
376	4734	0.606663	0.606663	-0.606663
377	4735	0.779785	-0.779785	0.779785
378	4736	0	0	0

Figure 10-23. Inter-Class Report.

As usual, the icons in the Inter-Class Report toolbar allow you to save, print or export your report, and navigate through the data on the gels. The *Settings* icon is used to customize your table display (for example, show the annotations in the master gel for all matches listed in your table). You can also create additional label categories in the master gel and update any modifications made to labels by using the *Annotate* and *Update Gel* buttons. The items in the *Histograms* icon are used to produce an Inter-Class Histogram or an Inter-Class + Intra-Class Histogram. Finally, you can generate related reports with the *Reports* icon:

- The **Gel Report** item can be used as a legend for the classes and their gels.
- The **Intra-Class Report** displays an Intra-Class Report (see Section 10.2.4) on the selected matches for a selected class.
- The **Report from Selection** function creates a new report that only contains the lines that are selected in the active report.

10.3.5 Statistical tests

ImageMaster provides three statistical tests: **two-sample t test**, **Mann-Whitney U test** and the **Kolmogorov-Smirnov test**. These tests are used to analyze differences in protein expression between classes of gels. The idea is to draw conclusions about the significance of the protein expression changes by extrapolating information from the data you collected. For example, when you have two samples (classes) with different means (that is, different means for the spot values of a particular match), you might want to know whether the data were sampled from populations with different means or whether the populations have the same mean with the observed difference being a coincidence of random sampling.

In fact, there is no way to definitely conclude which of the two possibilities is true. All you can do is calculate the probability of observing a certain difference (or larger) between sample means in an experiment of this size, for populations that in reality have the same mean. If the probability is small, you can conclude that the difference is not likely to be caused by random sampling and assume instead that the populations have different means.

NOTE! ImageMaster provides qualitative indications about different protein expressions. In order to state the results as probabilities, the data points (protein values in the case of ImageMaster) must respect the restrictive assumptions made by the various statistical tests (see below). If an assumption is not met even approximately, the significance levels and the power of the test are invalidated. Matters are made even worse when several assumptions are violated. Since this is often the case in studies of protein expression, ImageMaster only displays the numerical values for each test (and not the probabilities) and lets the user search in the appropriate tables for the corresponding significance levels. If the appropriate test was chosen and the assumptions were met, the probabilities found can be used and the results accepted. If this is not the case, the numerical values given by ImageMaster still allow you to classify the differences between the sample means according to their relative significance.

Two-sample t test

The two-sample t test is generally used to determine whether the mean (or median) of a variable differs between two populations. It is based on the following assumptions:

- The data are continuous (not discrete).
- The data follow the normal (Gaussian bell-shaped) probability distribution.
- The variances of the two populations are similar.
- The two samples are independent. There is no relationship between the individuals in one sample as compared to the other (as there is in a paired t test).

The t test statistic is based on the difference between the mean values ($\bar{X}$) of the two classes, normalized by the standard deviation (s). For this test, the number of degrees of freedom equals the total sample size ($n_1 + n_2$) minus 2. The t ratio is calculated as follows:

$$t = \frac{(\bar{X}_1 - \bar{X}_2)\sqrt{n_1 + n_2 - 2}}{\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)(n_1 s_1^2 + n_2 s_2^2)}}$$

If the t ratio is larger than a certain threshold, chosen according to a confidence level and the sample size, you can conclude that the difference between the two populations is statistically significant.

NOTE! Only the t ratio is given, and not the probability p of not being wrong when concluding that the two populations have significantly different means. This is for reasons of consistency and to make the user more aware of the implications of his or her analysis. In numerous cases, the restrictive assumptions are not met and the statistics will not be valid. Nevertheless, this in no way restricts the utility of the t test results, notably for classifying the spot differences by their potential significance. You should always check your hypotheses by visual inspection of the spots since results may be due to artifacts in the spot detection and/or matching steps.

If the data for the samples to be analyzed come from populations whose distribution violate the assumption of normality, then nonparametric tests like the Mann-Whitney or Kolmogorov-Smirnov tests can provide a better analysis.

Mann-Whitney or Wilcoxon test

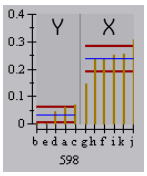
As indicated above, the Mann-Whitney U test or rank sum test is the nonparametric substitute for the two-sample t test when the assumption of normality is not valid. It is equivalent to the Wilcoxon rank sum test. Once again, it should only be used for comparing two unpaired samples. The assumptions of the Mann-Whitney U test are:

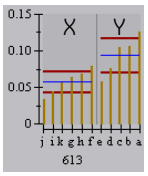
- The variable of interest is continuous (not discrete) and the measurement scale is at least ordinal. This means that repeated values (ties) are not acceptable. When ties are present in your data, there is an approximation provided in the calculations, but the exact results no longer hold.
- The distributions of the two samples are identical (although not necessarily normal) and differ only in location (that is central tendency).
- The two samples are independent.

To perform the Mann-Whitney test, ImageMaster first ranks all the spot values from low to high, paying no attention to which of the two classes (for example, X and Y) each value belongs. Then each value is given a rank number. The smallest number gets a rank of 1. The largest number gets a rank of N, where N is the total number of spot values in the two classes. If two values are the same, then they both get the average of the two ranks for which they tie. Finally, the ranks in each class are summed, thus giving W_X and W_Y, which are used to calculate the Mann-Whitney test statistic, U. The formula for U_X is as follows (The formula for U_Y is obtained by replacing X by Y):

$$U_X = W_X - \frac{n_X(n_X+1)}{2}$$

In fact, the smaller of the two calculated U values corresponds to the number of shifts needed in order that the spot values from the two populations do not overlap. For the first example in Figure 10-24, no shifts are necessary since the spot values of classes X and Y are already separated. On the contrary, for the second example the Mann-Whitney test indicates that the first two values from Y (or the three last values from X) have to be swapped four times in order to separate the samples (Figure 10-24).





Spot values	Ranks		Spot values	Ranks	
	X	**Y**		X	**Y**
0.000		**1.5**	0.034	1	
0.000		**1.5**	0.045	2	
0.044		**3**	0.056	3	
0.059		**4**	**0.058**		**4**
0.068		**5**	0.064	5	
0.144	6		0.069	6	
0.237	7		**0.075**		**7**
0.240	8		0.078	8	
0.249	9		**0.104**		**9**
0.255	10		**0.106**		**10**
0.308	11		**0.126**		**11**
W_X	51	15	W_X	25	41
n_X	6	5	n_X	6	5
U_X	30	**0**	U_X	**4**	26

Figure 10-24. Two examples to illustrate the Mann-Whitney and Kolmogorov-Smirnov tests. In (a) the two classes do not overlap at all, whereas in (b) 4 shifts are needed to completely separate the spot values of the two classes. The bold values correspond to the spot values and ranks from class Y, the others belong to class X.

In fact, ImageMaster displays the smaller of the two calculated U values in the Statistical Tests report. The lower this number, the higher the probability is that the means of the two samples are different. Knowing this value, and the sample size, you can easily look up the probabilities in a Mann-Whitney table.

Caution should be used when analyzing the results of a Mann-Whitney test. First, the assumptions are often violated. This is the case, for example, when spots are completely absent in one of the classes (in that case you have repeated values of 0). Moreover, if you have small samples, the Mann-Whitney test is meaningless. In fact, if the total sample size is seven or less, the test always gives a probability (of finding different means, in the case of identical populations) greater than 0.05, no matter how little the samples differ.

Kolmogorov-Smirnov test

The Kolmogorov-Smirnov test tries to determine if two data sets differ significantly. In other words, it is used to test whether or not two samples may reasonably be assumed to come from the same distribution. It has the advantage of not making an assumption about the distribution of the data and is frequently preferred over the Mann-Whitney rank sum test where there are many ties (repeated values). Note however, that this generally comes at a price. Other tests (for example, the t test) may be more sensitive if the data meet the requirements needed for that test. The assumptions of the Kolmogorov-Smirnov test are:

- The probability distributions are continuous.
- The measurement scale is at least ordinal.
- The two samples are mutually independent.

In the Kolmogorov-Smirnov test, the data points in each sample (spot values for a particular match in a class) are sorted in ascending order and converted into an empirical distribution function (EDF). This function gives the fraction of data points to the left of a given value z. In the second example from Figure 10-24, the ordered data points from class X are: 0.034, 0.045, 0.056, 0.064, 0.069 and 0.078. The fraction of data points to the left of each of these z values can easily be calculated and plotted (full line) in an Empirical Distribution Plot (Figure 10-25):

It is clear that no data lie strictly below 0.034, 17% = 0.17 = 1/6 of the data is strictly smaller than 0.045, 33% = 0.33 = 2/6 of the data is strictly smaller than 0.056, 50% = 0.50 = 3/6 of the data is strictly smaller than 0.064, and so on.

The same procedure can be followed for the second sample (class Y in our example, the dashed line in Figure 10-25). The Kolmogorov-Smirnov test statistic D is then defined as the maximum distance between the empirical distribution functions (EDF) of the two samples. In the example cited here, D is 0.63 (0.83-0.20). If D is greater than a particular decision limit (critical value found in a Kolmogorov-Smirnov table), there is a statistically significant difference between the two samples. However, the test provides no insight as to what causes the difference.

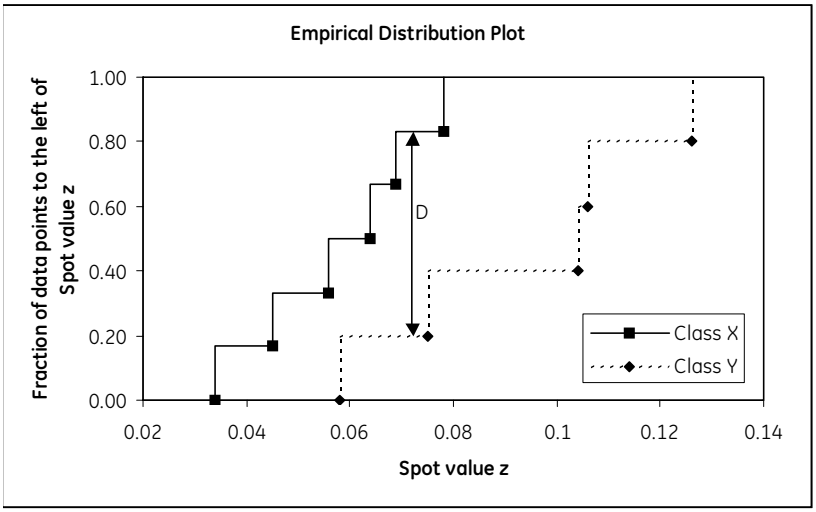


Figure 10-25. The empirical distribution plot for the spot values of match 613 (Figure 10-24), in Classes X and Y. The Kolmogorov-Smirnov statistic D corresponds to the maximum distance between the two empirical distribution functions.

Statistical tests report

The Statistical Tests report (Figure 10-26) contains, for each selected match, the desired statistical values, which quantify the differences between the means of two classes. These values should be considered as qualitative indications of the variations in protein expression between two populations, and only rarely (in ideal cases) should be used to calculate probabilities and draw quantitative conclusions. In addition, one should always check the results by visual inspection of the spots, since the conclusions may be erroneous due to inaccuracies in the detection or matching steps.

To obtain a report of statistical test results:

1 Select the matches to be studied (on the gels or on a report).

2 Select the gels to be included in the analysis.

3 Choose *Analyze* > *Inter-Class* > *Statistical Tests*.

4 In the pop-up list, select the value type (Intensity, Area, Vol, ...) to be displayed.

5 Choose one or more of the statistical tests for which you would like to see the results displayed.

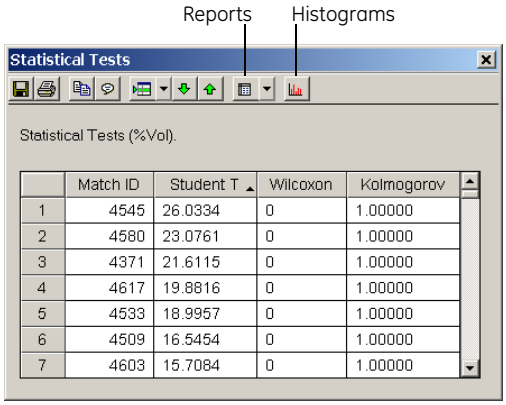


Figure 10-26. Statistical tests report.

The icons in the Statistical Tests report toolbar allow you to save, print or export your report, and navigate through the data on the gels. The *Histograms* icon is used to produce Inter-Class Histograms. Related reports can be selected from the *Reports* drop down menu:

- The **Gel Report** item can be used as a legend for the classes and their gels.
- The **Report from Selection** function creates a new report that only contains the lines that are selected in the active report.

NOTE! In order to focus your analysis on the most significant spot differences between classes, you can sort the values in the report by clicking on the column headers. This way, you can re-select the match that meet your own criteria of statistical value.

NOTE! The given statistical values are useless when the samples (classes) do not consist of more than two gels. In any event, your objective should always be to work with the largest possible sample sizes.

11 Data integration

11.1 Converting into ImageMaster 2D Platinum format

11.1.1 From earlier versions of ImageMaster and Melanie

ImageMaster recognizes gel files that were saved with ImageMaster 2D Platinum (4.9 or 5) and Melanie (2, 3, 4 or 5). If you add files from these versions in the Workspace Gels folder, you will recover spots and annotations, but no match data. However, you can convert entire analysis sets (such as a single folder containing all matched gels) into the current ImageMaster file format. This way all match information is recovered without having to redo the gel matching.

To convert ImageMaster 2D Platinum or Melanie data:

1 Choose *File > Import > ImageMaster 2D Platinum or Melanie Data* in the menu.

2 In the *Batch Files Conversion* box, indicate the **Source Folder** containing your gels and associated match files in one of the old format, by browsing to the desired directory.

3 Provide a **Destination Folder** name where your analysis set should be saved in ImageMaster 2D Platinum format. You can manually add the name of a new subfolder, if needed.

4 Click *OK*.

ImageMaster then creates new gels and match files in ImageMaster 2D Platinum 6.0 format and saves them to the given destination folder. These files are now ready to be added to the Workspace in ImageMaster.

11.1.2 From ImageMaster 2D Elite

Any 2D experiments from ImageMaster 2D Elite (or Phoretix 2D Elite) can continue to be analyzed by the new ImageMaster 2D Platinum project.

It is important to note that you can convert and use spot shapes and quantification values, matching information, 1D and 2D calibration results, annotations, spot filters, and much more from the Elite versions. However, you are not allowed to edit imported spots or compare quantification values from imported gels with new data obtained from the ImageMaster 2D Platinum software. This is because spot detection and especially spot quantification are done very differently in the two software versions, thus rendering any comparisons inappropriate. You can still compare your old gels with a new set of gels analyzed by the latest software, although you must redetect the spots and

redo the matching. All other information such as annotations and 1D or 2D calibrations are preserved.

To convert ImageMaster 2D Elite experiments:

1 In ImageMaster 2D Elite, make sure you have synchronized the spot numbers. This is necessary to correctly export the match information.

2 Export the experiment in XML format (by choosing *File* > *Import/Export XML* > *Save Experiment* from the ImageMaster 2D Elite menu). Save the XML file in the same folder as your experiment.

3 Start ImageMaster 2D Platinum 6.0 and choose *File* > *Import* > *ImageMaster 2D Elite Experiment* in the menu.

4 Browse the folder containing your Elite experiment, and select the saved XML file.

5 You are asked to enter the **Source Folder** containing the experiment gels (indicated by default if you saved your XML file in the experiment folder) as well as the **Destination Folder** for saving the new project in ImageMaster 2D Platinum format. Note that you can create a new subfolder by adding the name manually.

6 Click *OK* to confirm your folder settings.

7 The import operation takes a few seconds.

ImageMaster then creates new gels and project files in ImageMaster 2D Platinum 6.0 format and saves them to the given destination folder. These files are now ready to be added to the Workspace in ImageMaster.

Elite experiments
All gels in the original experiment are converted into the ImageMaster 2D Platinum format and inserted into a new workspace and project bearing the same name as the experiment (see Chapter 4). The generated project can contain several match sets, depending on whether or not the initial experiment contained average gels. A separate match set is created for each Average Gel.

User Defined Fields in an experiment are converted to properties of a project and stored in the project file. However, they are currently not supported by the software. The Comments field in the Experiment Header Information is converted to a Comment for the project.

Gel images
The gel images (originally in .tiff, .gel or .img format) are treated as 16-bit TIFF files and inserted in the newly created project folder. In the case of an .img file, an

accompanying .inf file should be present in the experiment folder. When the .inf file is missing, a warning message is displayed and import is aborted. In that case, try creating a valid .inf file (resolution and gel size information must be known) and do the import of the experiment again.

If the images were initially calibrated, the calibration is also applied to the converted gel images. Similarly, if the Invert Intensities option in the Elite Experiment was turned on, the TIFF image is inverted upon conversion of the gel.

If a master gel was defined in the original experiment, this status is also maintained in the new master file (tagged in red).

The Name, Title, Comments and User Defined Fields in the Gel Header box of the Elite Experiment are transferred to Gel Properties in the ImageMaster 2D Platinum files.

Average gels
In the ImageMaster 2D Platinum project, an average gel from the Elite Experiment becomes the master of a match set that contains the gels that were composing it.

Spots
Converted spots have spot shapes and quantification values extracted from the experiment file. As spot areas and the associated volumes in ImageMaster 2D Elite are calculated in terms of pixels (and not mm^2), the **Compute area in mm2** box in the *Quantification* tab of the ImageMaster *Options* (accessible by choosing *Tools* > *Options* in the menu) is not checked to reflect this fact. Please note that it is prohibited to edit spots from imported files. If you need to compare imported gels with new ones, you must do an automatic spot detection in ImageMaster 2D Platinum.

Comments on spots (previously visible in the Edit Spot Fields box) are converted to spot annotations of the Comment category. Any User Defined Fields for spots (entered through the Edit Spot Fields box or Measurements window) are converted to annotations of a category with the name of the original User Defined Field. Note that User Fields of type Document are converted to annotations that contain a file link (the label content is file:file path).

Filtered spots (not displayed in the Elite version) are imported into ImageMaster 2D Platinum, but are flagged with annotations of type Set:Filtered_Spot. You can easily select these spots (*Select* > *Annotations* > *By Category*) and decide if you want to keep, delete or hide them. Similarly, all spots that were selected for picking are flagged with annotations of type Set:Picked_Spot.

Matches
If gels were matched in the original experiment, the spot matches are converted into ImageMaster 2D Platinum matches.

1D and 2D calibrations
1D calibration steps are converted to annotations of the pI_MW category. For pI calibrations, the labels contain the pI value, followed by a space and the number -1 (indicating that no MW information was available for this annotation). For MW calibrations, the labels contain the number -1 (indicating that no pI data was available), followed by a space and the MW expressed in Daltons (Da). Using this data, ImageMaster 2D Platinum automatically displays computed pI and MW values for all the spots in the gel (and any matched gels).

2D calibration information, available from the Protein List in ImageMaster 2D Elite, is also converted to annotations of the pI_MW category when proteins are assigned to spots. In this case, the labels contain the pI value, followed by a space and the MW expressed in Da.

Protein lists
Information from the Protein Lists in ImageMaster 2D Elite is converted to spot annotations. The pI and MW values are transferred to annotations of the pI_MW category (see above). Accession numbers appear under the Ac category.

Annotations
Annotations from ImageMaster 2D Elite are converted to annotations (of category Annotation). Please note that annotation formats such as font, style, size, color, etc. are lost.

11.1.3 From Twain compatible scanners

ImageMaster can also acquire images directly from TWAIN compatible scanners. You must indicate the scanning source (all TWAIN compatible scanners attached to your PC are automatically recognized by ImageMaster) and then launch the scan. The scanner software opens, giving you the opportunity to change the necessary settings, and subsequently initiate the scanning process. Once this is done, the image is saved in ImageMaster file format and can be added to the Workspace.

To scan one or more gel images using a TWAIN compatible scanner:

1 Choose *File* > *Import* > *Twain* > *Select Source* in the menu.

2 Select the appropriate image capture device from the given list. This only needs to be done once (unless you want to change to a new image capture device).

3 Choose *File* > *Import* > *Twain* > *Acquire* in the menu.

4 The scanner software automatically starts.

5 Set the suitable parameters for the scan.

6 Launch the scan.

11.2 Exporting and importing ImageMaster 2D Platinum data

11.2.1 XML format

To make the vast amounts of data that can be obtained with ImageMaster available for processing by other applications or to import information coming from external sources, ImageMaster uses the common XML format. XML is also exploited for saving reports.

XML stands for eXtensible Markup Language and was created as a cross-platform, software and hardware independent tool to structure, store, and exchange information. It allows the creation of customized tags, enabling the definition, transmission, validation, and interpretation of data between applications and organizations.

XML files can be viewed in the latest versions of Internet Explorer (version 5 or higher), Netscape (version 6 or higher) and Mozilla (version 1.4 or higher). However, as XML was designed to describe data and not to display data, it does not look like a web page. An XML document contains color-coded root and child elements. A plus (+) or minus sign (-) to the left of the elements can be clicked to expand or collapse the element structure. If you want to view the raw XML source, you must select *View* > *Source* from the browser menu.

XML does not use predefined tags, as is the case for HTML. Therefore, the browser does not understand the meaning of the tags and does not know how to display the XML document. Therefore, XSL (eXtensible Stylesheet Language) stylesheets must be used in addition to the XML document to transform the XML into the sort of document that is recognized by the browser. This is the case when tabular reports, or a history or script are printed from ImageMaster. The software uses the XSL stylesheets located in the Template folder of the ImageMaster installation directory to print attractive documents. If you are familiar with XML and XSL, you can even create personalized templates for printing. Notice that XSL stylesheets can also be used to convert an XML file into another XML file.

NOTE! Because the XSL stylesheets are specific to the browser you use, you will find that different versions (both for printing reports and scripts) are installed with the software (in the Template\Report and Template\Script folders of the installation directory). ImageMaster will therefore ask you to choose the appropriate XSL template each time you print a report, script or history. Look at which template works with your browser, and delete the other one. In this way, the software automatically opens the remaining file and does not ask you to make a choice.

11.2.2 ImageMaster reports

The default mode for saving reports in ImageMaster is in XML format. Reports saved in this format carry the extension .rpt and are restored to their original tabular form, on the condition that they were inserted into a Workspace. When reopened in ImageMaster, these reports can be manipulated, sorted, customized and edited as with any newly displayed report.

Outside the ImageMaster program, the content of ImageMaster reports can be viewed with a browser. However, only a color-coded raw view of the root and child elements is available (generally not very useful). The main interest in XML format, besides being used directly by the ImageMaster software, is that external applications can easily extract necessary data. Moreover, the files can be converted to other user-defined formats.

Please note that the classical reports in ImageMaster contain particular data types, and to export the comprehensive information of an analysis set, the creation of several reports would often be necessary. This is why ImageMaster offers an additional feature for exporting gel data.

11.2.3 Export gel data

ImageMaster allows the export of all gel-related data, except for the gel image itself, into a single XML file. This XML file can include all available information on a set of gels, together with spots (shape, quantification, aligned coordinates), annotations, and match information (Figure 11-1).

To export gel data to an XML file:

1 Select all objects (gels, spots, annotations) that should be exported to an XML file. No preliminary selection is necessary if all gels in the active worksheet should be exported, potentially with all their spot, annotation and match data.

2 Choose *File* > *Export* > *Gel Data to XML* in the menu.

3 Select the data to be exported from the *Export Gel Data to XML File* dialog. See below for more details about the items that can be exported.

4 If objects are selected, ImageMaster asks whether you want to save only the selected objects or not. If you answer *Yes*, just the selected objects are exported. *No* means that you want to export all information on all open gels.

5 Enter a name and destination folder for the file to be generated.

6 ImageMaster creates a file on the hard disk that can be read by other applications. You can also view the contents of the file with your browser.

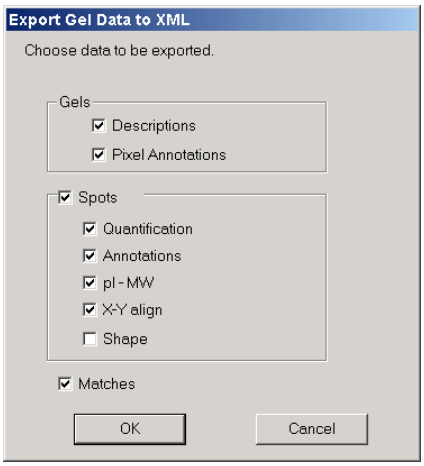


Figure 11-1. Export Gel Data to XML File dialog box.

The following items can be exported to the XML file:

Gels: Minimal exported items for gels are gel IDs, gel names, and summarized information about gels as present in the Gel Report. This includes image height and width in pixels, pixel dimensions of the scanned image, minimum and maximum gray levels (raw and calibrated), intensity calibration parameters, number of spots, number of annotations, gel class and match set assignments. In addition, the following data can be added to the exported file:

- **Descriptions**: User-defined gel descriptions, as entered in the Gel Description Report.
- **Pixel Annotations**: Exports all annotations placed on individual gel pixels.

Spots: Minimal exported properties for spots are X and Y coordinates on the gel, and Spot ID. In addition, the following data can be added to the exported file:

- **Quantification**: All quantification values as appearing in the Spots Report.
- **Annotations**: Annotations linked to spots.
- **pI - MW**: Calculated pI and MW values, if available.
- **X-Y align**: Aligned spot coordinates, if the gels were aligned.
- **Shape**: Spot shape descriptions using directional chain codes.

Matches: Exports all match information between all or selected spots in the exported files.

11.2.4 Import gel data

Similar to the export function, gel data can be imported from an XML file previously generated by ImageMaster or produced by another application (the file should contain the appropriate tags, recognized by ImageMaster):

To import gel data:

1 Select a single gel you want to input data from an XML file.

2 Choose *File* > *Import* > *Gel Data from XML* from the menu.

3 Browse the appropriate folder and pick the desired file name.

4 If data from several gels is present in the file, ImageMaster asks you to specify the gel from which data should be imported.

5 Select the data to be imported from the *Import Gel Data from XML File* dialog box. See below for more details about the items that can be imported. Some options may be inactivated if the necessary information is not present in the opened file.

6 ImageMaster imports the desired information.

The following items can be imported from an XML file (Figure 11-2):

Gels: You can import the following data from existing gels:

- **Calibration**: Intensity calibration parameters (Slope and Offset).
- **Descriptions**: User-defined gel descriptions, entered in the Gel Description Report.

Spots: Imports spots. Note that spot shape descriptions using directional chain codes need to be present in the file.

Annotations: Imports both pixel and spot annotations.

NOTE! When several types of objects are imported at the same time, all import operations should take place correctly. If this is not the case (because of ambiguous or erroneous file formatting), none of the objects are imported.

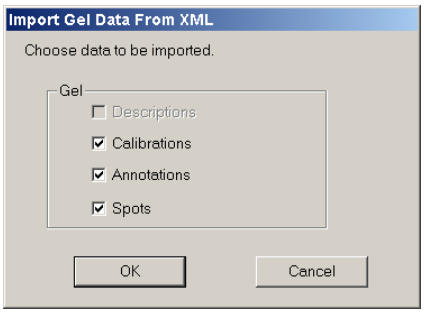


Figure 11-2. Import Gel Data from XML File dialog box.

11.3 Exporting to spot excision robots

Please note that in addition to exporting spots to an excision robot, it may be useful to annotate the exported spots. This allows you to easily select them later on, for adding experimental data (such as mass spectrometry information).

11.3.1 Bruker Proteineer SP spot picker

ImageMaster can export spot coordinates directly to the Bruker Proteineer SP spot picker. For more details about this functionality, please see the documentation provided by Bruker Daltonics.

To export a spot coordinate file to the Bruker Proteineer SP:

1 Select all spots to be cut with the spot excision robot.

2 Choose *File* > *Export* > *Spots to Picker* > *Bruker Proteineer SP* in the menu.

11.3.2 GE Healthcare Ettan spot picker

To use the Ettan Spot Picker, two adhesive markers should be placed on the gel before scanning. These markers are used for the calibration of the coordinates, that is, for determining the correspondence between the X and Y positions of the

analyzed gel image and the coordinates of the actual gel located on the spot picker (Figure 11-3).

Once the gel has been digitized and analyzed with ImageMaster 2D Platinum, the software can generate a pick list. This list contains the location, in pixels, of the center of each spot you wish to pick, as well as the pixel coordinates of the centers of the two reference markers.

To export a file with spot coordinates for use by the spot picker, you first have to open the image files and perform image analysis (spot detection is mandatory). You should then annotate the reference markers. If a reference marker is well detected during the automatic spot detection process (nice round spot perfectly centered on the marker), you can just add an annotation on the marker spot. The basis of such an annotation is displayed as a small square, and its coordinates correspond to the center of the spot. If a reference marker is not well detected (irregular shape or consisting of several spots), it is better to delete the existing spot(s) and create an annotation on the pixel that is in the center of the marker. This kind of pixel annotation has a crossed basis and its coordinates correspond to the pixel it is attached to. Note that the two options can be used in a single gel (one marker with a spot annotation and the other with a pixel annotation), as long as the annotations are centered on the markers.

To create a pick list:

1 Identify the two reference markers on your image.

2 Zoom the image to better see the left reference marker.

3 Check if the marker is detected as a nice, round spot. If it is not, select the spot(s) on the marker and delete by choosing *Edit* > *Spots* > *Delete* from the menu.

4 Click on the *Annotation* tool in the main toolbar.

5 Double click on the marker spot, or if such a spot is not present, on the pixel that is in the middle of the marker.

6 Select the **Comment** category.

7 Enter **IR1** as the label text.

8 If an annotation attached to a pixel is not in the center of the reference marker, you can move it to the appropriate position. Do this by clicking on its basis (cross) and holding down the left mouse button while dragging the annotation to its new position.

9 Move your gel to see the second (right) reference marker.

10 Repeat the procedure, but enter the label text **IR2** this time.

11 Once the two markers have been annotated, select spots to pick (Figure 11-3).

12 Choose *File* > *Export* > *Spots to Picker* > *GE Healthcare Ettan*.

13 For each gel, you will be asked to save a pick list in text or XML format (only the text file can be read by the Ettan Spot Picker).

14 The Ettan Spot Picker can now read the exported files.

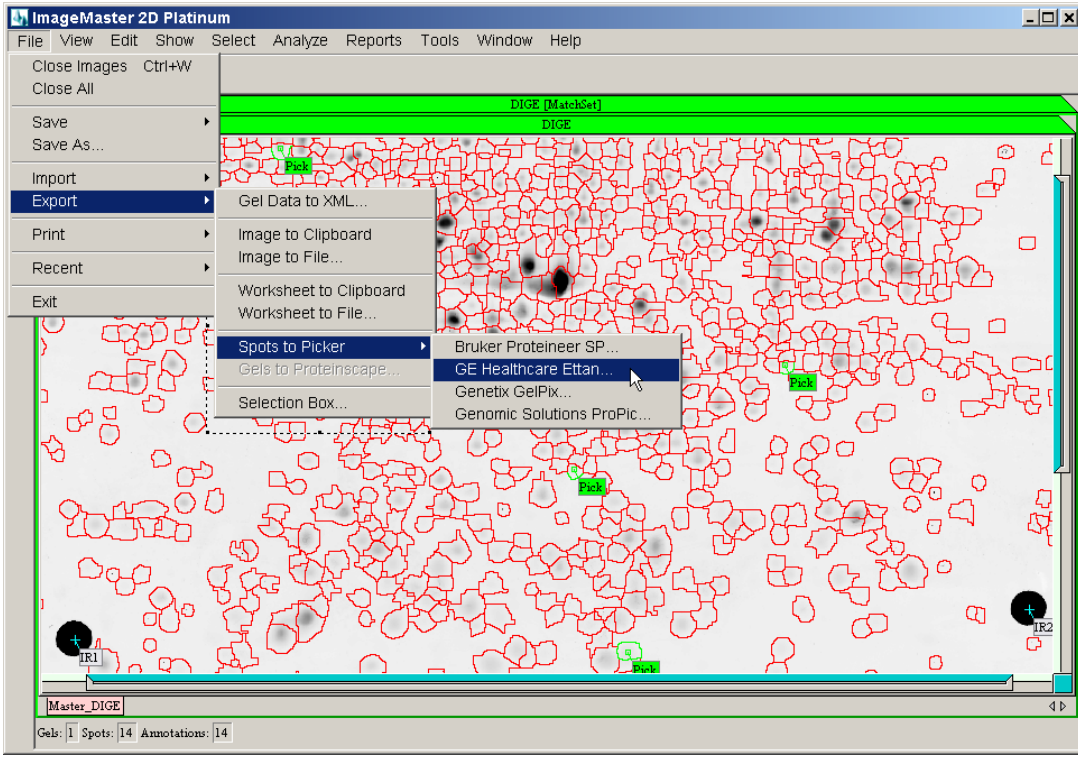


Figure 11-3. Reference markers IR1 and IR2. IR1 is attached to a pixel (cross basis). IR2 is attached to a spot. Both options can be used in a single gel. Spots to be picked are selected (highlighted in green).

11.3.3 Genetix GelPix spot picker

To export a spot coordinate file to the GelPix spot picker:

1 Select all spots to be cut with the spot excision robot.

2 Choose *File* > *Export* > *Spots to Picker* > *Genetix GelPix* in the menu.

3 Enter a file name and destination folder.

4 The spot picker subsequently reads the saved file.

11.3.4 Genomic Solutions ProPic spot picker

The ProPic spot picker produces a TIFF file of the entire gel holder area with a resolution of 1035 x 1317 pixels, each pixel representing approximately 330 x 330 microns of the image area. This ProPic image can be analyzed with ImageMaster, and spot coordinates in the image can be exported back to the ProPic spot picker in a predetermined file format. The ProPic software subsequently translates the image X, Y coordinates into ProPic robot X, Y coordinates by using a robot map. Since the robot mapping process assumes that each X, Y coordinate in the image always corresponds to the same position on the robot bed, the ProPic image must remain in its original form. It can never be resized, cropped or rotated.

The ProPic image is a picture of the current state of the gel from which the spots are to be picked. It is not necessarily the image used to determine the spots to pick. One can select spots from any analytical image (of the same gel) obtained from a different resolution system and analyzed with ImageMaster. However, the analytical image needs to be aligned to the ProPic image so that ImageMaster can export the aligned X and Y coordinates of the selected spots. Please keep in mind that proper alignment is crucial at this stage, especially for images of "old" gels that underwent significant shape change since they were originally imaged and analyzed, and for gel images that were acquired on a high-resolution system.

To export a spot coordinate file to the ProPic spot picker:

1 Analyze your analytical gel image with ImageMaster and select the spots you want to export to the spot picker. Annotate these spots (with a category of type Set:) so that you can easily re-select them at a later stage.

2 Place your gel on the ProPic robot bed, produce the ProPic image and open it in ImageMaster. Use the gel image as is, meaning do not resize, crop, flip or rotate the image.

3 Align your analytical gel image to the ProPic image, using a sufficient number of landmarks (see Section 6.3.6 for details on how to align gel images).

4 Select all spots to be cut with the spot excision robot from the analytical image.

5 Make sure only the analytical gel image is selected.

6 Choose *File* > *Export* > *Spots to Picker* > *Genomic Solutions ProPic*.

7 Enter a file name and destination folder. The file automatically is given a **.tds** extension (TwoDSpotlist).

8 ImageMaster exports the aligned X and Y coordinates for each selected spot. That is, the coordinate system of the ProPic image will be used.

9 The spot picker subsequently reads the saved file.

11.4 Connecting to external protein databases

A key advantage of the ImageMaster program is its ability to link spots on gel images to protein data in 2-DE or other databases. 2-DE databases contain information on proteins identified on 2-DE images, such as pI and MW values, bibliographical references to protein related literature, information on protein functions, etc. ImageMaster can work in conjunction with several specialized 2-DE databases, but is particularly compatible with data from the SWISS-2DPAGE database that is accessible over the Internet. If your computer has access to the World Wide Web, you can remotely query and retrieve protein data related to spots on your gels.

NOTE! The ImageMaster software provides access to several databases on the Internet. It is the responsibility of the user to acquire the database licenses, if needed. In particular, the PROSITE and SWISS-2DPAGE databases are copyrighted, and all commercial users of these databases are required to purchase a database license from GeneBio. No license fee is charged to academic users for non-commercial use. For questions about obtaining a license subscription for the PROSITE and SWISS-2DPAGE databases, please contact GeneBio (www.genebio.com).

11.4.1 Setting the database

A detailed description of how to query a remote database through the Internet is given in Chapter 8. In short, these queries use the label (holding a protein accession number taken from a 2-DE database) of a particular category (linked to a specific database) to interrogate the database. The HTTP query must be composed of:

- The **database HTTP address** (for example, http://www.expasy.org/).

- The **database query engine** (for example, cgi-bin/nice2dpage.pl?).
- The **database accession number** (for example P02649).

The database HTTP address and query engine are entered as constraints to the annotation category. Go to the *Categories* tab in the ImageMaster *Options* (accessible by choosing *Tools* > *Options* in the menu), and type them in as one string in the **External Engine** field (for example, http://www.expasy.org/cgi-bin/nice2dpage.pl?). A list of federated 2-D PAGE databases, with the required database query formats, can be found at http://www.expasy.org/ch2d/2d-access.html.

11.4.2 Querying the database

The database accession number (for example P02649) is entered as a label of the particular category linked to a spot. It identifies the protein corresponding to that spot.

When you subsequently double click on the label while the *Annotation* tool is selected, ImageMaster opens your default Internet browser and launches a query that consists of the concatenation of the HTTP address, query engine and accession number (for example, http://www.expasy.org/cgi-bin/nice2dpage.pl?P02649). As a result, the entry for the protein with the given accession number opens in your browser (Figure 11-4). In the case of the above example, this would be the entry for Human Apo E (Gels).

11.4.3 SWISS-2DPAGE master gels

SWISS-2DPAGE master gels, or reference maps, contain annotations for all proteins corresponding to entries in the SWISS-2DPAGE 2-DE database. Each annotation contains the accession number (in the Ac category) of the database entry, as well as the entry's short name (in the ProteinName category). For example, the Human Plasma protein map possesses the Ac label P02768 and the ProteinName label ALBU_HUMAN, for each spot that corresponds to albumin.

In most 2-DE databases the accession number is the unique entry identifier, while the protein name can vary. It is therefore strongly recommended to use accession numbers for your queries. Nevertheless, ImageMaster provides a way to automatically update the protein names in your gels, if you use the SWISS-2DPAGE accession numbers.

To update ImageMaster labels with database protein names:

1 Select a gel containing labels with valid SWISS-2DPAGE accession numbers.

2 Select all labels you want to update.

3 Choose *Edit* > *Annotations* > *Load Protein Name from SWISS-2DPAGE*.

4 For each selected label, ImageMaster then requests its short name from the SWISS-2DPAGE database (using the Ac as identifier) and then updates the ProteinName label accordingly.

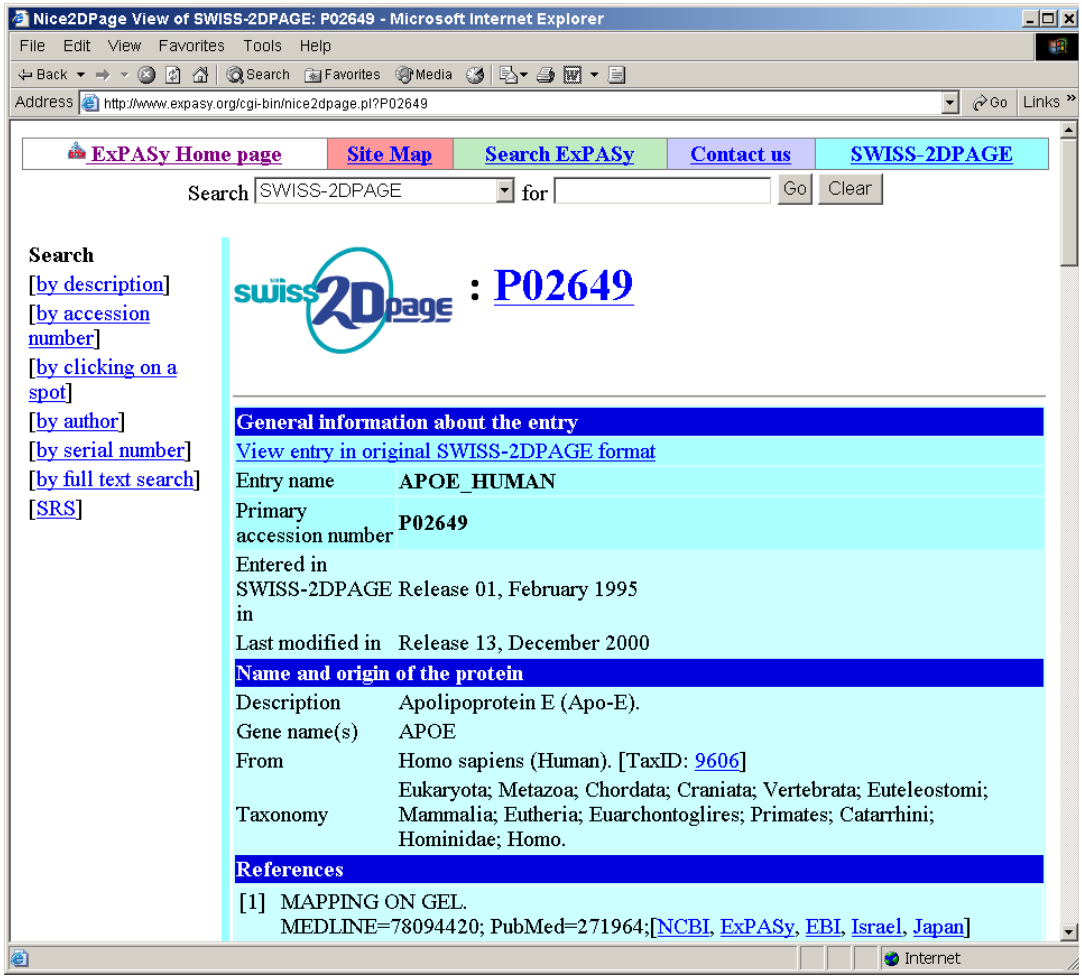

Nice2DPage View of SWISS-2DPAGE: P02649 - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Address http://www.expasy.org/cgi-bin/nice2dpage.pl?P02649
ExPASy Home page
Site Map
Search ExPASy
Contact us
SWISS-2DPAGE
Search SWISS-2DPAGE for Go Clear
Search
[by description]
[by accession number]
[by clicking on a spot]
[by author]
[by serial number]
[by full text search]
[SRS]
swiss2Dpage : P02649
General information about the entry
View entry in original SWISS-2DPAGE format
Entry name APOE_HUMAN
Primary accession number P02649
Entered in SWISS-2DPAGE Release 01, February 1995
Last modified in Release 13, December 2000
Name and origin of the protein
Description Apolipoprotein E (Apo-E).
Gene name(s) APOE
From Homo sapiens (Human). [TaxID: 9606]
Taxonomy Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini; Hominidae; Homo.
References
[1] MAPPING ON GEL.
MEDLINE=78094420; PubMed=271964;[NCBI, ExPASy, EBI, Israel, Japan]
Internet

Figure 11-4. The SWISS-2DPAGE entry for human Apo E. Entries from this database contain full protein names, bibliographic references, annotations (such as protein function, pathological variations) and the pI and MW of the related spots on the 2-DE maps. It also includes cross-references to numerous other databases.

12 Safety, control and automation

12.1 Introduction

Discover how ImageMaster lets you monitor the operations carried out on your images and automate various analysis steps in this chapter. Although the three options associated with these features - namely **Undo/Redo**, **History** and **Script** - may seem quite independent, they in fact share a number of common interface elements and properties that are described below.

12.1.1 Action descriptors

Each operation carried out in ImageMaster is described by a generic name called Action Descriptor. The Action Descriptor is used in Undo/Redo, History and Script to identify a specific operation so that you can act on it (for example, undo the operation or copy it from a history to a new script). Nearly all operations executed are displayed in the Action Descriptor list, which you can find in the Undo/Redo, History and Script windows. However, there are some exceptions to this depending upon the option used. These exclusions are specified in the ensuing sections.

12.1.2 Displayed actions

The number and type of operations included in the Undo/Redo lists and History can become very large and diverse, thus making it difficult to find the actions of interest (when deleting or copying to a new script, for instance). In order to reduce the number of displayed actions, four major types of operations were defined:

- **Edition**: Covers the functionalities available under the *Edit* menu, this kind of operation renders permanent modifications to the image data.
- **Selection**: Comprises the options from the *Select* menu. These allow the selection of specific objects or the refinement of an existing selection. Once selected, you can perform further actions on the chosen objects.
- **Display**: This type of operation is typically found in the *View*, *Analyze*, *Report* and *Window* menus. It generally displays new windows (for example reports), modifies the Display Zone (layout or stack settings) or manages the visual relationships between images (for example alignment).
- **Show**: The features available from the *Show* menu belong to this type of operation. They act as a kind of visual filter or can be considered as non-permanent modifications to the gels because nothing is saved with the image file.

All ImageMaster functions are classified into one of these types so that three different filter criteria can be applied to the items in the Action Descriptor list.

These filter criteria and the type of actions they include are listed in the table below:

	Edition	Selection	Display	Show
All	X	X	X	X
Without Display	X	X		
Only Edit	X			

Each of these filters has a specific context:

- **All** is interesting if you want to list all types of operations carried out during your work session.
- **Without Display** is particularly useful when generating scripts. Modifications to the display (move image, etc.) are not important and often cannot be scripted.
- **Only Edit** is used when you want to track permanent changes that have been made to the images and image data.

An additional filter can be combined with any of the above three filters. When the **Only Gels Action** box is checked, only actions that operate on selected images are displayed. Thus, you can exclude operations that act at the level of the program window (for example, layout settings) or that result in the generation of reports. By default, this box is not checked and therefore all operations (in the filter types above) are listed.

12.1.3 History and script navigators

The Action Descriptor lists in the History and Script windows have the form of a navigator, in which some Action Descriptors are preceded by a + or - node allowing the item to be expanded or collapsed, and an icon indicates whether it can be scripted or not:

	The corresponding action does not require any user-dependent input (such as the selection of a region) and can therefore be **scripted**.
	Actions identified by this icon **cannot be scripted** because they require user intervention. Even if selected in the History navigator, they are not copied to any newly-generated script.

Once an action is expanded, you see one or both of the following icons:

	Identifies a **Parameter**. This represents a major property of the action. For instance, the **Select Rows from Report** action is characterized by a **Window** parameter (describing the title and type of the report), the **Sort** parameter (indicating the column in which the report data was sorted), and the **Selection** parameter (containing information about which items in the report were selected).
	Identifies an editable **Value** that characterizes an action or an action parameter.

12.1.4 Gel selection

The listed actions only include the operations relevant to the gel images selected when first opening the History window or activating the Undo or Redo functions. So even if the gel selection changes and the History is refreshed, the Action Descriptors only apply to the originally selected gels. Therefore, if you want a history for one or more images that were not selected during the creation of the current history, you must select the image(s) in question and open a new History window. Similarly, if you want to undo a general action (for example, spot detection) on specific images, you should first select the desired gels and then choose the Undo function from the menu.

12.2 Undo / redo

ImageMaster allows you to cancel any unwanted modifications. You can return to an earlier state of your analysis by using the Undo option (Figure 12-1). You no longer lose whole parts of your analysis simply because you made an error and could only recover a previously saved state. Moreover, ImageMaster not only offers a multiple Undo function, it also enables you to restrict the canceled actions to specific images. Please keep in mind that actions can only be undone for images that were selected when the operation was originally performed, even if the gel selection subsequently changed.

Each Action Descriptor in the Undo/Redo list is preceded by the time at which the action was carried out. This information helps you to identify the particular sequence of actions that you would like to cancel.

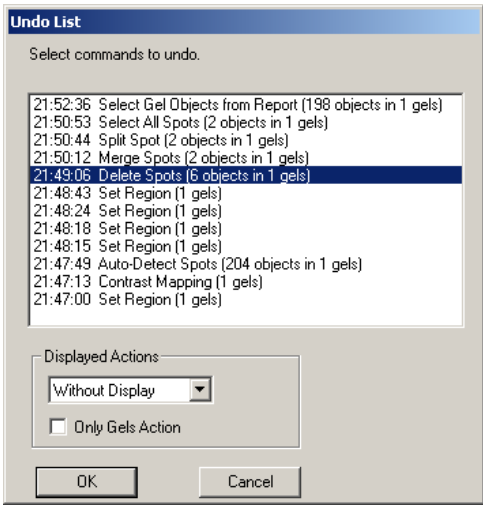

Undo List
Select commands to undo.
21:52:36 Select Gel Objects from Report (198 objects in 1 gels)
21:50:53 Select All Spots (2 objects in 1 gels)
21:50:44 Split Spot (2 objects in 1 gels)
21:50:12 Merge Spots (2 objects in 1 gels)
21:49:06 Delete Spots (6 objects in 1 gels)
21:48:43 Set Region (1 gels)
21:48:24 Set Region (1 gels)
21:48:18 Set Region (1 gels)
21:48:15 Set Region (1 gels)
21:47:49 Auto-Detect Spots (204 objects in 1 gels)
21:47:13 Contrast Mapping (1 gels)
21:47:00 Set Region (1 gels)
Displayed Actions
Without Display
Only Gels Action
OK
Cancel

Figure 12-1. Undo window.

To undo the last action performed:

1 Select the images for which you would like to cancel the most recent operation.

2 Choose *Edit* > *Undo* from the menu.

3 The last action is selected by default.

4 Click *OK*.

To undo several successive actions:

1 Select the images for which you would like to cancel a certain number of operations.

2 Choose *Edit* > *Undo* from the menu.

3 Select a prior action to be undone.

4 Click *OK*. The selected action and all following actions are undone automatically.

If you are not satisfied with your latest undo or you canceled too many operations, you can obviously reapply the actions.

To redo specific actions:

1 Select the images for which you would like to redo a certain number of canceled operations.

2 Choose *Edit* > *Redo* from the menu.

3 Select the action to be redone.

4 Click *OK*. The selected action and any preceding actions are redone.

All operations performed can be reversed with the exception of the following non-exhaustive list:

- Modifications to the Workspace cannot be canceled.
- It is not possible to undo operations such as opening, saving and printing files.
- Changes that only apply to all or none of the gels cannot be canceled (for example, displaying the profile or changing the color palette).
- Direct actions on reports (such as the creation, edition or closure) cannot be undone. However, when selections or modifications in a report are propagated to the corresponding images, they are included in the undo list and can therefore be canceled.

12.3 History

Please keep in mind that actions are only displayed for images that were selected when the History window was first opened, even if the gel selection subsequently changed and the History was refreshed.

To open a History window:

1 Select the images for which you would like to display a history.

2 Choose *Edit* > *History* > *Show* from the menu.

3 You can place a marker in the History by choosing *Edit* > *History* > *Insert Marker* from the menu, or clear the list of actions by choosing *Edit* > *History* > *Clear.*

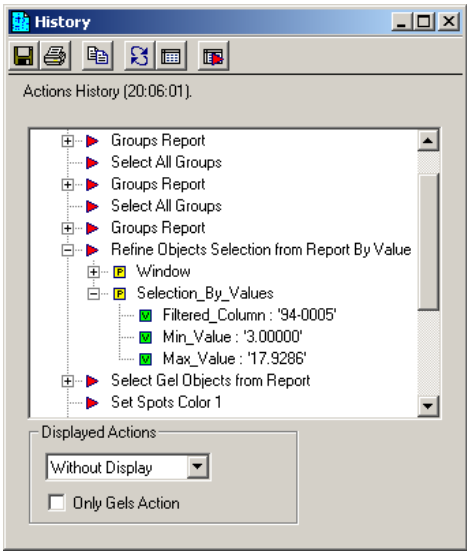


Figure 12-2. History window.

The History window contains a toolbar and a list of actions (Figure 12-2). The functionality of each of the icons is as follows:

	Save the History in an XML type file with extension .hst.
	Print the History. You can choose to print only selected actions. Note that before printing the History first displays in your default Internet browser. The XSL stylesheet located in the *Template\Script* folder of the ImageMaster installation directory is used to transform the XML formatted History into an attractive document (find more details about XML and XSL in Chapter 11). You can then use the print option in your browser to get a paper copy.
	Copy the selected actions for subsequent pasting into an open script.
	Refresh the History. All actions are collapsed and the list scrolls down until only the most recent operations are visible.
	Display the **Gels Report**. Note that the report only includes the images selected when the History window was first opened. This tool is useful for checking which images are included in the current History.
	Copy the selected actions to a **New Script**.

Similar to the Undo/Redo options, there are some actions that are not included in the History. The following is a non-exhaustive list of exceptions:

- Modifications applied to the Workspace are not logged.
- Operations that require file manipulations such as opening, saving and printing files are currently not recorded with the History function.
- Direct actions on reports are not included in the History navigator, except when selections or modifications in the report are propagated to the corresponding images.

12.4 Script

The Script function automates parts of the analysis process. ImageMaster routinely carries out a sequence of instructions when you run a script. You are empowered to encode desired actions simply by cutting and pasting from the History to the Script windows, without ever needing any programming knowledge (Figure 12-3). Several features in ImageMaster make it possible to modify a script to best suit your immediate requirements.

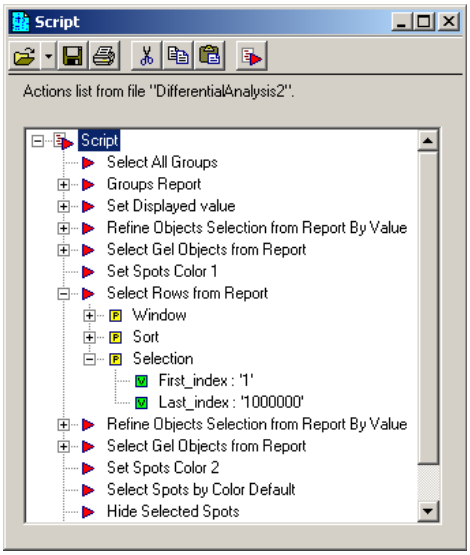


Figure 12-3. Script window.

To create a script:

1 Carry out the desired sequence of operations on a selected set of images.

2 Choose *Edit* > *History* > *Show* to display the *History* window.

3 Copy the required actions to a new script by selecting the actions and clicking the *New Script* icon in the *History* window toolbar.

4 A *Script* window appears on your screen.

The Script window contains a toolbar and a list of actions. The functionality of each of the icons is as follows:

	Open an existing script. Browse the directory where the script file (.srt) is located and select the file name. The drop down menu gives you access to a list of scripts opened during the current work session and scripts that were previously saved in the default *ImageMaster\Scripts* folder in the user's *My Documents* directory.
	Save the script. You are given the choice of saving only the selected actions. Scripts saved in the default *ImageMaster\Scripts* folder in the user's *My Documents* directory can be accessed directly using the *Open* icon.
	Print the script. Choose to print only the selected actions. Note that before printing the script is first displayed in your default Internet browser. The XSL stylesheet located in the *Template\Script* folder of the ImageMaster installation directory is used to transform the XML formatted script into an attractive document (find more details about XML and XSL in Chapter 11). You can then use the print option in your browser to get a paper copy.
	Cut the selected actions from your script.
	Copy the selected actions for subsequent pasting in a script.
	Paste the previously copied actions (from any *History* or *Script* window) below the currently selected action.
	Run the script. Be sure to select all the images that should be run with the script.

The originally encoded script sometimes needs to be modified because you forgot to do an operation, would like to suppress an action, or want to adapt some parameters to a specific set of gels. The following tools and possibilities are available for modifying your script:

- Move the actions in a script by dragging and dropping them into another position. They are placed below the point of your cursor.
- Copy actions from any history or script using the *Copy* tool and paste them into your script using the *Paste* tool. Pasted actions are inserted at the end of your script or below the currently selected action, but can be moved simply by dragging and dropping them into another position.
- Suppress one or several actions in your script by selecting the actions and clicking the *Cut* icon.
- Modify the values that characterize the actions or action parameters. To do so, right click on the **Value** icon and choose *Modify* from the contextual menu. Enter a new value.
- Oblige ImageMaster to ask for user input while the script is running. It is practical, for example, if you want to postpone the choice of statistics until the moment you actually run the script. To do this, right click on the **Action Descriptor** and choose *Set User Action* from the contextual menu. The script will stop at the designated point and display a standard dialog box relating to the operation, thus enabling you to adjust your input. Once all the user definitions are made, click the *OK* button and the script will continue to run.

The *Tools* menu in the ImageMaster program menu bar offers additional functionality for creating or handling scripts.

To create a script:

1 Choose *Tools* > *Script* > *New* from the menu.

2 Code your new script by copying specific actions from a history or other script using the tools described above.

3 Save your new script using the corresponding icon in the *Script* window toolbar.

To run an existing script:

1 Choose *Tools* > *Script* > *Run* from the menu.

2 Browse the directory where the script file is located and select its name.

3 ImageMaster may ask you for approval to run the script (see below). Answer *Yes*.

4 The script runs immediately, stopping at each operation that was set as a user input action or when an illogical or impossible operation is

encountered (for example, Select All Matches when images were not matched).

To require confirmation before running a script:

1 Go to *Tools* > *Script* > *With Confirmation* in the menu. If *With Confirmation* is checked, then the option is activated. This is the default setting.

2 To deactivate this option, choose *Tools* > *Script* > *With Confirmation*. *With Confirmation* is no longer checked in the menu.

To run a recently used script :

3 Go to *Tools* > *Script* > *Recent* in the menu.

4 Choose one of the scripts from the list (only possible if scripts were used or created during the current work session).

5 The script runs immediately (ImageMaster may ask for confirmation).

A Appendix

A.1 Contextual menus

Contextual menus are available throughout the Workspace and Display Zone. In the Workspace, right click on a file or folder to open a contextual menu from which you choose an action to be carried out. In the Display Zone, access contextual menus by right clicking worksheet or pane banners, gel legends or inside a gel image. These contextual menu options are often duplicates of main menu options but are an alternative and quicker way to perform the action.

A.2 Workspace contextual menus

A.2.1 Workspace name

- **New Project**: Creates a project in the workspace.
- **Insert Project**: Adds an existing project to the workspace.
- **Backup**: Archives the workspace file (with the extension .bkp).
- **Restore Workspace**: Returns an archived workspace to its previous state.
- **Restore Project**: Returns an archived project to its previous state.
- **Properties**: Displays the workspace properties: Workspace Name, Creator, File Name, Modification Date and Comment.

A.2.2 Project name

- **Export**: Exports a project with copies of its gels, matches and documents.
- **Backup**: Archives the project file (with the extension .bkp).
- **Delete**: Removes the project from the active workspace.
- **Properties**: Displays the project properties: Project Name, Creator, File Name, Modification Date and Comment.

A.2.3 Gels folder

- **Add Gels**: Adds gels that are already in ImageMaster format (with the extension .mel).
- **Import Gels**: Adds new gels that are not already in ImageMaster format.
- **Create Folder**: Creates a subfolder into the Gels folder.

- **Paste**: Pastes previously cut or copied items into the Gels folder.

Folder

- **Open > In Worksheet**: Opens all gels contained in the folder in the active [Gels] worksheet in the Display Zone.
- **Open > In New Worksheet**: Opens all gels contained in the folder in a new [Gels] worksheet in the Display Zone.
- **Add Gels**: Adds gels that are already in ImageMaster format (with the extension .mel).
- **Import Gels**: adds new gels that are not already in ImageMaster format.
- **Create Folder**: Creates a subfolder into the selected folder.
- **Delete**: Removes the selected folder.
- **Cut**: Cuts the selected folder in order to paste it.
- **Paste**: Pastes previously cut or copied items into the selected folder.
- **Properties**: Displays the folder properties: Name and Comment.

Gel

- **Open > In Worksheet**: Opens the selected gels in the active [Gels] worksheet in the Display Zone. You can also double click on a file to open it in the active [Gels] worksheet.
- **Open > In New Worksheet**: Opens the selected gels in a new [Gels] worksheet in the Display Zone.
- **Add in Matchset**: Adds the selected gels in an existing match set or in a new one. If you would like to create a new match set, enter a new name. Otherwise, you can choose from the other match sets available in the proposed list.
- **Delete**: Removes the selected gels from the folder. Please note that it does not remove the file from your hard disk. The image is only deleted from the active project.
- **Search**: Searches for the gel on the hard disk (in the indicated directory) when a gel with the appropriate ID is not found at the usual location. This is the case when a red mark covers the gel icon, generally indicating that you moved the original gel file or replaced it with another one.
- **Cut**: Cuts the selected gels in order to paste them.
- **Properties**: Displays the gel properties: Gel Name and Staining.

A.2.4 DIGE Gels folder

- **Add DIGE Gel**: Adds DIGE images that are already in ImageMaster format (with the extension .mel) to define a DIGE gel.
- **Import DIGE Gel**: Adds DIGE images that are not already in ImageMaster format to define a DIGE gel.
- **Paste**: Pastes previously cut or copied items into the DIGE Gels folder.

DIGE gel

- **Open > In Worksheet**: Opens the selected DIGE gels in the active [Gels] worksheet in the Display Zone. You can also double click on a file to open it in the active worksheet.
- **Open > In New Worksheet**: Opens the selected gels in a new worksheet in the Display Zone.
- **Detect**: Performs spot detection on the selected DIGE gels. You are asked to estimate the Number of Spots to be found in the gels.
- **Create MatchSet**: Creates a new match set that will include the selected DIGE gels as sub match sets. The name of the DIGE gel is input as the default Name.
- **Delete**: Removes the selected gels from the folder. Please note that it does not remove the file from your hard disk. The DIGE gel is only deleted from the active project.
- **Copy**: Copies the selected gels into memory. You can then paste them into other folders of your workspace.
- **Properties**: Displays the DIGE properties: Name, Dye Chemistry and Comment.

DIGE gel image

- **Open > In Worksheet**: Opens the selected DIGE images in the active [Gels] worksheet in the Display Zone. You can also double click on a file to open it in the active [Gels] worksheet.
- **Open > In New Worksheet**: Opens the selected DIGE images in a new [Gels] worksheet in the Display Zone.
- **Histogram**: Shows data associated with detected spots in two selected images from the same DIGE gel. Spot data (intensity, area, volume or slope) is plotted against log volume ratio. The blue curve represents the frequency distribution of the log volume ratios.

- **Add in Matchset**: Adds the selected DIGE images in an existing match set or in a new one. If you would like to create a new match set, enter a new name. Otherwise, you can choose from the other match sets available in the proposed list.
- **Search**: Searches for the gel on the hard disk (in the indicated directory) when a gel with the appropriate ID is not found at the usual location. This is the case when a red mark covers the gel image icon, generally indicating that you moved the original image file or replaced it with another one.
- **Copy**: Copies the selected images into memory.
- **Properties**: Displays the DIGE gel properties: Gel Name and Staining.

A.2.5 MatchSets folder

- **Create MatchSet**: Creates a new match set into the MatchSets folder.
- **Paste**: Pastes previously cut or copied items into the MatchSets folder.

MatchSet

- **Open**: Opens the selected match set in a new [MatchSet] worksheet.
- **Create MatchSet**: Creates a sub match set within the selected match set.
- **Delete**: Removes the selected match set from the folder.
- **Copy**: Copies the selected match set into memory.
- **Cut**: Cuts the selected match set in order to paste it.
- **Paste**: Pastes previously cut or copied items into the selected match set.
- **Properties**: Displays the match set properties: MatchSet Name and Comment.

SubMatchSet

- **Open**: Opens the selected match set in a new [MatchSet] worksheet.
- **Set MatchSet as Reference**: Defines the selected match set as the reference to be used when matching two or more match sets. The match set icon is tagged red.
- **Create MatchSet**: This option is available only if the sub match set is empty, It creates a sub sub match set within the selected sub match set.
- **Delete**: Removes the selected match set from the folder.
- **Copy**: Copies the selected match set into memory.

- **Cut**: Cuts the selected match set in order to paste it.
- **Paste**: Pastes previously cut or copied items into the selected match set.
- **Properties**: Displays the match set properties: MatchSet Name and Comment.

Gel

- **Open**: Opens the selected gels with the corresponding master in a new [MatchSet] worksheet.
- **Set Gel as Reference**: Defines the selected gel as the reference to be used when matching two or more gels. The gel icon is tagged red. A master gel is automatically created by duplicating the reference gel when opening the match set in a worksheet.
- **Delete**: Applies to non-DIGE images only. Removes the selected gels from the match set. Please note that it does not remove the file from your hard disk. The image is only deleted from the active project.
- **Add in Classes**: Adds the selected gels in a class. If you would like to add the gels to a new class, then enter a new name. Otherwise, you can choose from the other classes available in the proposed list.
- **Copy**: Copies the selected gels into memory.
- **Properties**: Displays the gel properties: Gel Name and Staining.

A.2.6 Classes folder

- **Create Class**: Creates a new class in the Classes folder.
- **Paste**: Pastes previously cut or copied items into the Classes folder.

Class

- **Open**: Opens the selected class in a new [Classes] worksheet.
- **Create Class**: Creates a subclass within the selected class.
- **Delete**: Removes the selected classes from the Classes folder.
- **Cut**: Cuts the selected classes in order to paste them.
- **Paste**: Pastes previously cut or copied items into the selected class.
- **Properties**: Displays the class properties: Name and Comment.

Gel

- **Open**: Opens the selected gels in a new [Classes] worksheet.

- **Delete**: Removes the selected gels from the class. Please note that it does not remove the file from your hard disk. The gel is only deleted from the class in the active project.
- **Copy**: Copies the selected gels into memory.
- **Cut**: Cuts the selected gels in order to paste them.
- **Properties**: Displays the gel properties: Gel Name and Staining.

A.2.7 Reports folder

- **Open**: Opens all the reports contained in the Reports folder in dockable windows.
- **Add Reports**: Inserts one or more reports from the hard disk into the Reports folder.
- **Create Folder**: Creates a subfolder within the Reports folder.
- **Paste**: Pastes previously cut or copied items into the Reports folder.

Folder

- **Open**: Opens all the reports in dockable windows.
- **Add Reports**: Inserts one or more reports from the hard disk into the selected folder
- **Create Folder**: Creates a subfolder within the selected folder.
- **Delete**: Removes the selected folder.
- **Copy**: Copies the selected folders into memory.
- **Cut**: Cuts the selected folders in order to paste them.
- **Paste**: Pastes the previously cut or copied folders or reports in the selected folder.
- **Properties**: View or edit the Name or Comment for the folder.

Report

- **Open**: Opens the selected reports in dockable windows. You can also double click on a report file to open it.
- **Delete**: Removes the selected reports from the folder. Please note that it does not remove the file from your hard disk. The report is only deleted from the active project.

- **Search**: Searches for the report on the hard disk (in the indicated directory) when a report with the appropriate ID is not found at the usual location. This is the case when a red mark covers the report icon, generally indicating that you moved the original report file or replaced it with another one.
- **Copy**: Copies the selected reports into memory.
- **Paste**: Pastes the previously cut or copied items below the selected report.
- **Properties**: Displays the report properties: Path, Type, Message, and Comment. The Type item tells you whether your report is a Spot Report, Gel Report, Match Report, etc. The Message gives information on the spot value or statistics that were used to generate the report, or other data depending on the report type. The Comment field displays the report comment that was defined by the user.

A.2.8 Documents folder

- **Open**: Opens all the files, contained in the Documents folder, with the appropriate software, depending on the extensions. As in Windows Explorer, files with unrecognizable extensions are not opened.
- **Add Files**: Inserts one or more files from the hard disk within the Documents folder.
- **Create Folder**: Creates a subfolder within the Documents folder.
- **Paste**: Pastes the previously cut or copied items in the Documents folder.

Folder

- **Open**: Opens all the files with the appropriate software, depending on the extensions. As in Windows Explorer, files with unrecognizable extensions are not opened.
- **Add Files**: Inserts one or more files from the hard disk within the selected folder.
- **Create Folder**: Creates a subfolder within the selected folder.
- **Delete**: Removes the selected folders (with their files).
- **Copy**: Copies the selected folders into memory.
- **Cut**: Cuts the selected folders in order to paste them.
- **Paste**: Pastes the previously cut or copied items in the selected folder.
- **Properties**: Displays the folder properties: Name and Comment.

File

- **Open**: Opens the selected files with the appropriate software, depending on the extensions. You can also double click on a single file to open it.
- **Delete**: Removes the selected files from the folder. Please note that it does not remove the file from your hard disk. The file is only deleted from the active project.
- **Copy**: Copies the selected files into memory.
- **Paste**: Pastes the previously cut or copied items below the selected file.
- **Properties**: Displays the file path.

A.3 Display zone contextual menus

A.3.1 Worksheet, pane or legend

- **Close Images**: Closes one or more selected gel images. This operation can also be carried out by selecting the applicable images and going to *File* > *Close Images* or press Ctrl+W.
- **Close All:** Closes all worksheets available in the Display Zone. This operation can also be carried out by going to *File* > *Close All*.
- **Save Worksheet**: Saves all the changes made to the gel images displayed in the active worksheet.
- **Pane**: Show Vectors, Hide Vectors, Stacked pane, Tiled pane.
- **Select**: All, Aligned, Inverse Selection.

A.3.2 Image

Gels

- **File**: Close Images, Close All, Save Worksheet.
- **Select**: All, Aligned, Inverse Selection.
- **Report**: Generates a Gel Report.

Spots

- **Edit**: Detect, Edit Enabled, Copy from Image, Delete.
- **Show**: Shape, Show All, Hide All, In Region, Only Selected, Hide Selected, Show ID, Hide All ID, Set Color.

- **Select**: By ID, By Color, In Region, From Report File, All, Refine by Value, Inverse Selection.
- **Report**: Generates a Spot Report.

Annotations

- **Edit**: Add Label, Modify, Copy, Paste Annotations, Paste Labels, Duplicate Labels, Delete, Delete by Category, Copy Matched Labels, Link with Spot, Unlink from Spot.
- **Show**: Show All, Hide All, In Region, For Spots, Only Selected, Hide Selected, Visible Categories, Packed Categories, Linked Data, Set Color.
- **Select**: By Content, By Category, Combine, By Color, In Region, From Report File, Common Labels, All, Inverse Selection.
- **Report**: Label Report, Annotation Report, Category Report.

Matches

- **Edit**: Add Match, Delete Match.
- **Show**: Show ID, Hide All ID, Show Vectors, Hide Vectors.
- **Select**: By ID, For Spots, All, Refine Selection, Inverse Selection, Multiple Matches.
- **Analyze**: Intra-Class Report, Intra-Class Histograms.
- **Match Report**: Generates a Match Report.

- **Zoom**: Zoom in or out on the selected images or regions. You can also choose a factor to reduce or enlarge the selected images or regions by.
- **Show All**: Makes all spots and annotations visible in the selected gel images.
- **Hide All**: Hides all spots and annotations in the selected gel images. It also hides all match vectors in the selected gel images.
- **Select All**: Activates all spots and annotations in the selected gel images.
- **Unselect All**: Deactivates all spots and annotations in the selected gel images.

B Appendix

B.1 Shortcut keys

Several menu options can be activated by keyboard shortcuts. These are indicated at the right-hand side of the corresponding menu option. Please note the logic behind the key combinations:

Ctrl is used for handling **gels**.

Shift is used for handling **spots**.

Alt is used for handling **annotations**.

Ctrl + Shift is used for handling **matches**.

Some exceptions do exist. The most important ones are the following two shortcuts used for undoing and redoing actions carried out on a gel.

Shortcut	**Menu Command**
Ctrl+Z	*Edit > Undo*
Shift+Z	*Edit > Redo*

B.2 Gel shortcuts

Shortcut	Menu Command
Ctrl+A	*Select > Gels > All*
Ctrl+F	*View > Switch*
Ctrl+I	*Show > Gels > Grid Lines > Show*
Ctrl+J	*Show > Gels > Grid Lines > Hide*
Ctrl+N	*Select > Gels > Inverse Selection*
Ctrl+S	*File > Save > Worksheet*
Ctrl+W	*File > Close Images*
Ctrl+<Up>	*Show > Gels > Move > Up*
Ctrl+<Down>	*Show > Gels > Move > Down*
Ctrl+<Left>	*Show > Gels > Move > Left*
Ctrl+<Right>	*Show > Gels > Move > Right*
Ctrl+1	*Tools > Toolbar > Hand*
Ctrl+2	*Tools > Toolbar > Magnify*
Ctrl+3	*Tools > Toolbar > Region*
Ctrl+4	*Tools > Toolbar > Spot*
Ctrl+5	*Tools > Toolbar > Annotation*
<Page Up>	*View > Pane Layout > Next*
<Page Down>	*View > Pane Layout > Previous*
Shift+<Up>	*Show > Gels > Zoom > In*
Shift+<Down>	*Show > Gels > Zoom > Out*
F1	*Show > Show Reference/Master*
F2	*Show > Show All*
F3	*Show > Hide All*
F4	*Select > Select All*
F5	*Select > Unselect All*

B.3 Spot shortcuts

Shortcut	Menu Command
Shift+A	*Select > Spots > All*
Shift+B	*Select > Spots > In Region*
Shift+E	*Edit > Spots > Edit Enabled*
Shift+H	*Show > Spots > Hide All*
Shift+I	*Show > Spots > Hide Selected*
Shift+J	*Show > Spots > Show ID*
Shift+K	*Show > Spots > Hide All ID*
Shift+N	*Select > Spots > Inverse Selection*
Shift+R	*Show > Spots > In Region*
Shift+T	*Show > Spots > Only Selected*
Shift+X	*Edit > Spots > Delete*
Shift+Y	*Show > Spots > Show All*
Shift+1	*Show > Spots > Shape > Crossed*
Shift+2	*Show > Spots > Shape > Outlined*
Shift+3	*Show > Spots > Shape > Filled*
Shift+4	*Show > Spots > Shape > Outlined/Filled*
Shift+5	*Show > Spots > Set Color > Purple*
Shift+6	*Show > Spots > Set Color > Orange*
Shift+7	*Show > Spots > Set Color > Brown*
Shift+8	*Show > Spots > Set Color > Default*

B.4 Annotation shortcuts

Shortcut	Menu Command
Alt+A	*Select > Annotations > All*
Alt+B	*Select > Annotations > In Region*
Alt+C	*Edit > Annotations > Copy*
Alt+D	*Edit > Annotations > Delete by Category*
Alt+E	*Edit > Annotations > Modify*
Alt+F	*Edit > Annotations > Add Label*
Alt+H	*Show > Annotations > Hide All*
Alt+I	*Show > Annotations > Hide Selected*
Alt+J	*Show > Annotations > Visible Categories*
Alt+K	*Show > Annotations > Packed Categories*
Alt+L	*Edit > Annotations > Link with Spot*
Alt+N	*Select > Annotations > Inverse Selection*
Alt+O	*Show > Annotations > For Spots*
Alt+P	*Edit > Annotations > Paste Annotations*
Alt+R	*Show > Annotations > In Region*
Alt+T	*Show > Annotations > Only Selected*
Alt+U	*Edit > Annotations > Unlink from Spot*
Alt+V	*Edit > Annotations > Paste Labels*
Alt+X	*Edit > Annotations > Delete*
Alt+Y	*Show > Annotations > Show All*
Alt+4	*Show > Annotations > Set Color > Purple*
Alt+5	*Show > Annotations > Set Color > Orange*
Alt+6	*Show > Annotations > Set Color > Brown*
Alt+7	*Show > Annotations > Set Color > Default*
Alt+8	*Select > Annotations > By Color > Purple*
Alt+9	*Select > Annotations > By Color > Orange*
Alt+0	*Select > Annotations > By Color > Brown*

B.5 Match shortcuts

Shortcut	Menu Command
Ctrl+Shift+A	*Select > Matches > All*
Ctrl+Shift+G	*Edit > Matches > Add Match*
Ctrl+Shift+H	*Show > Matches > Hide Vectors*
Ctrl+Shift+J	*Show > Matches > Show ID*
Ctrl+Shift+K	*Show > Matches > Hide All ID*
Ctrl+Shift+M	*Select > Matches > For Spots*
Ctrl+Shift+N	*Select > Matches > Inverse Selection*
Ctrl+Shift+U	*Edit > Matches > Delete Match*
Ctrl+Shift+Y	*Show > Matches > Show Vectors*

C Appendix

C.1 Software references

Appel RD, Hochstrasser DF, Roch C, Funk M, Muller AF and Pellegrini C (1988) Automatic classification of two-dimensional gel electrophoresis pictures by heuristic clustering analysis: A step toward machine learning. *Electrophoresis* 9: 136-142.

Appel RD, Hochstrasser DF, Funk M, Vargas JR, Pellegrini C, Muller AF and Scherrer J-R (1991) The MELANIE project - from a biopsy to automatic protein map interpretation by computer. *Electrophoresis* 12: 722-735.

Appel RD, Palagi PM, Walther D, Vargas JR, Sanchez J-C, Ravier F, Pasquali C and Hochstrasser DF (1997) Melanie II - a third generation software package for analysis of two-dimensional electrophoresis images: I. Features and user interface. *Electrophoresis* 18: 2724-2734.

Appel RD, Vargas JR, Palagi PM, Walther D and Hochstrasser DF (1997) Melanie II - a third generation software package for analysis of two-dimensional electrophoresis images: II. Algorithms. *Electrophoresis* 18: 2735-2748.

Appel RD and Hochstrasser DF (1998) Computer analysis of 2-D images. In: Link AJ (ed) *Methods in Molecular Biology, Vol 112: 2-D Protocols for Proteome Analysis*, pp 363-381. Totowa NJ: Humana Press.

ExPASy Molecular Biology Server (2003) The Melanie 2-DE analysis software. [Online] http://www.expasy.org/melanie.

Miller MJ, Olson AD and Thorgeirsson SS (1984) Computer analysis of two-dimensional gels: automatic matching. *Electrophoresis* 5: 297-303.

Pun T, Hochstrasser DF, Appel RD, Funk M, Villars-Augsburger V and Pellegrini C (1988) Computerized classification of two-dimensional gel electrophoretograms by correspondence analysis and ascendant hierarchical clustering. *Applied and Theoretical Electrophoresis* 1: 3-9.

Vargas RJ (1996) Two-dimensional gel electrophoresis computer analysis systems: from image acquisition to protein identification. *Ph.D. thesis*, Faculty of science, Geneva University.

Wilkins MC, Hochstrasser DF, Sanchez J-C, Bairoch A and Appel RD (1996) Integrating two-dimensional gel databases using the Melanie II software. *Trends in Biochemical Sciences* 21: 496-497.

C.2 Statistical methods

Armitage P and Berry G (1987) Statistical methods and medical research. Oxford, London: Blackwell Scientific Publications.

Kim JO and Mueller CW (1978) Introduction to factor analysis: What is and how to do it? Newbury Park: Sage Publications.

Tabachnick B and Fidell LS (1996) Using multivariate statistics (3rd edition). New York: Harper Collins College Publishers.

C.3 Further reading

Appel RD, Bairoch A, Sanchez J-C, Vargas JR, Golaz O, Pasquali C and Hochstrasser DH (1996) Federated 2-DE database: a simple means of publishing 2-DE data. *Electrophoresis* 17: 540-546.

Appel RD, Sanchez J-C, Bairoch A, Golaz O, Ravier F, Pasquali C, Hughes GJ and Hochstrasser DF (1996) The SWISS-2DPAGE database of two-dimensional polyacrylamide gel electrophoresis. *Nucleic Acids Research* 22: 3581-3582.

Binz PA, Mueller M, Walther D, Bienvenut WV, Gras R, Hoogland C, Bouchet G, Gasteiger E, Fabbretti R, Gay S, Palagi P, Wilkins MR, Rouge V, Tonella L, Paesano S, Rossellat G, Karmime A, Bairoch A, Sanchez JC, Appel RD and Hochstrasser DF (1999) A molecular scanner to automate proteomic research and to display proteome images. *Analytical Chemistry* 71: 4981-4988.

Binz PA, Wilkins MR, Gasteiger E, Bairoch A, Appel RD and Hochstrasser DF (1999) Internet resources for protein identification and characterization. In: Kellner R, Lottspeich F, Meyer HE (eds) Microcharacterization of Proteins, 2nd ed., pp. 277-300. Weinheim: Wiley-VCH.

ExPASy Molecular Biology Server (2003) [Online] http://www.expasy.org.

Hoogland C, Baujard V, Sanchez J-C, Hochstrasser DF and Appel RD (1997) Make2ddb: a simple package to set up a 2-DE database on the WWW. *Electrophoresis* 18: 2755-2758.

Hoogland C, Sanchez J-C, Bairoch A, Hochstrasser DF and Appel RD (1999) The SWISS-2DPAGE database: what has changed during the last year. *Nucleic Acids Research* 27: 289-291.

Link AJ (ed) (1998) *Methods in molecular biology, Vol 112: 2-D Protocols for Proteome Analysis*. Totowa NJ: Humana Press.

Lopez MF (2000) Better approaches to finding the needle in a haystack: optimizing proteome analysis through automation. *Electrophoresis* 21: 1082-1093.

Sanchez J-C, Wilkins M, Appel RD and Hochstrasser DF (1997) Identifying proteins for proteome studies. In: Creighton ET (ed) *Protein Function: a practical approach,* 2nd ed., pp. 1–27. IRL Press.

Wilkins MR, Williams KL, Appel RD and Hochstrasser DF (eds) (1997) *Proteome research: new frontiers in functional genomics*. Berlin Heidelberg: Springer Verlag.

Unlu M, Morgan ME, and Minden JS (1997) Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* 18: 2071–2077.

Tonge R, Shaw J, Middleton B, Rowlinson R, Rayner S, Young J, Pognan F, Hawkins E, Currie I and Davison M (2001) Validation and development of fluorescence two-dimensional differential gel electrophoresis proteomics technology. *Proteomics* 1: 377–396.

Index

Numerics
2-DE gel electrophoresis 7
3D view
display axes 106
synchronize all 106

A
Ac category 152
accession number 152
action descriptors 249
activate ImageMaster 18
adapt gradations
inter-class histograms 222
intra-class histograms 204
adjust contrast 96
analyze menu 23, 65
annotation 12
add 171
add labels 153
combine sets 163, 165
copy 171
create 152
create label categories 154
delete 174
display 167
flag position 167
flagpole color 168
hide 168
import 175
link with spot 154
modify 171
pack categories 163, 170
report 176, 177
select 161
select by category 162
select by content 162
show 168
tool 24, 161, 171
auto hide 29
automatic spot detection 128

B
backup workspace 62, 63
batch files conversion 233
Bruker Proteineer SP spot picker 241

C
calculated
MW 143
pI 143
calibration 106

control ... 110
export graph ... 110
open ... 109
print graph ... 110
report ... 110
save ... 109
strip ... 106
category ... 151
create ... 174
create sets ... 158
data type ... 155
external engine ... 156
is unique ... 156
rename ... 173
report ... 176, 177
central tendency ... 199
class ... 12, 54
add gels in workspace ... 57
create in workspace ... 56
heuristic clustering ... 215
open ... 58
properties ... 59
comment category ... 152
contextual menus ... 28
gel image ... 266
legend ... 266
pane banner ... 266
worksheet banner ... 266
contrast mapping ... 96
control
calibration ... 110
cursor information ... 95, 143

D

data analysis ... 195
differential gel electrophoresis ... 8
DIGE ... 8
add gels to workspace ... 44
histogram ... 148
import gels in workspace ... 43
internal standard ... 10
report ... 150
dispersion ... 200
display zone ... 24
displayed actions ... 249
dockable ... 28
windows ... 28
documents ... 60
add files to workspace ... 61
create folder in workspace ... 60
open ... 61

E

edit

menu 23
edit spots 139
eLicensing 18
exit ImageMaster 21
export 237
gel data to XML 238

F

factor analysis 209
interpret 210
report 209
file
link 159
menu 23
fitting report 110, 196
floating 29
floating license 18
folder
properties 45
further reading 276

G

GE Healthcare Ettan spot picker 241
gel 11
add to workspace 43
align 92
anatomy 11
calibrate 106
calibration report 122
create folder in workspace 42
crop 118
delete 114
description report 120
erase 114
export 124
gray levels 96
grid lines 90
IDs 61
import in workspace 42
invert gray levels 117
load image data 94
move 82
open 45
print 125
properties 46
rename 114
rotate 115
synthetic 191
unload image data 94
zoom 83
gels folder 41
Genetix GelPix spot picker 244
Genomic Solutions ProPic spot picker 244
graphical user interface 22

grid lines 90

H

hand tool 24, 82
hardware requirements 17
help
- menu 23
- online 2
- user manual 2

heuristic clustering 214
histograms
- inter-class 220
- inter-class and intra-class 222
- intra-class 201

history 253
- copy to new script 254
- navigator 250
- print 254
- refresh 254
- save 254

http link 159

I

ImageMaster
- 2D Elite 233
- 2D Platinum 5.0 233
- DIGE 15
- modules 15
- window 22

images
- close 27
- depth 80
- format 79
- resolution 79
- select 27
- stack 27

import 237
- gel data to XML 240

installation 17
intensity normalization 112
inter-class
- histograms 220
- overlapping measures 216
- report 178, 224
- specify 216
- statistics 216

intra-class
- histograms 201
- normalize 204
- report 190, 205

isoelectric point calibration 142

K

keyboard shortcuts 23

L
label 12
add 171
copy 172
delete 174
display 167
duplicate 173
import 175
modify 171
report 176
select 161
landmark
annotations 183
category 152
legend 27
link objects to external data 158

M
magnify tool 24, 83
master image 179
match 12, 179
add 188
delete 188
display 186
hide 187
ID 187
refine selection 184
report 189
select 183
sets 13
show 187
show ID 187
statistics report 190
vectors 187
matching 179
annotations 183
automatic 181
landmarks 183
start 182
matchsets 46, 179
add gels in workspace 51
copy and paste 51
create in workspace 49
master image 179
open 52
properties 53
reference image 52, 179
measure histogram 146
Melanie 233
menu bar 23
min area 131
molecular weight calibration 142

N
next 27
node-locked license 18
normalization 106, 111, 204

O
outliers 208

P
panes 26
  close 26
  select 26
  stack 26
pI_MW category 143, 152
pinned 28
population matching 179
predefined label categories 151
previous 27
profile 101
projects 39
  add 40
  create 39
  insert 40
  open 40
  properties 40
  view 35
protein databases 245
  query 156, 246
pseudo colors 100

Q
query databases 156

R
redo 251
reference
  maps 246
region tool 24, 81
regions 11
regular expressions 163
report 59
  3D view 65
  add to workspace 59
  create folder in workspace 59
  export 238
  gel 65
  gel calibration 65
  gel description 65
  IDs 61
  inter-class 224
  open 60
  statistical tests 230
  toolbar 67
reports menu 23, 65

representative spots 208
restore workspace 63

S
saliency 130
scatter
plot 112, 196
report 196
script 255
create 256
navigator 250
open 256
print 256
run 256
save 256
select
menu 23
next 68
on gels 68
previous 68
selection box
export 118
import 119
set category 152
setup wizard 17
shortcuts 269
annotations 272
gels 270
matches 273
spots 271
show menu 23
smooth 130
software
features 1
references 275
requirements 17
sort
by similarity 205
values in inter-class histograms 222
values in intra-class histograms 205
spot 12
add 141
automatic detection 129
borders 128
color 137
delete 142
delete part 140
detection 127
detection parameters 130
display 136
edit 138
export 241
filter 136
hide 137

ID 127, 138
increase size 139
merge 141
pickers 241
preview of detection 128
report 145, 208
save detection parameters 130
select 135
shape 136
show 137
split 140
tool 24, 135
spot filtering 192
start ImageMaster 21
statistical tests 225
Kolmogorov 229
Mann-Whitney 227
report 230
Smirnov 229
two-sample t test 226
Wilcoxon 227
statistics
descriptive 198
inter-class 216
intra-class 196
methodology 276
status bar 28
step tablet 106
control 110
SWISS-2DPAGE gels 246
synthetic gels 191
create 193
system requirements 17

T

tabbed groups 30
text link 161
three-dimensional view 102
toolbar 24
tools menu 23
Twain compatible scanners 236

U

undo 251
unique identifiers 61
un-pinned 29
UUIDs 61

V

view menu 23

W

window menu 23
workflow 13, 33

worksheets 25
close 26
rename 25
select 25
workspace
archive 62
backup 62, 63
classes 54
create 36
documents 60
gel icons 34
gels folder 41
hierarchy 37
matchsets 46
navigator 35
open 36
properties 39
reports 59
restore 63
save 36
structure 37
toolbar 35
window 35

X

XML format 237
XSL stylesheet 237

Z

zoom window 85

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